A Secondary
BOOK of BIOLOGY

4. *Bacteriology and Hygiene*

M. CHINERY

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4. Bacteriology and Hygiene

By M. Chinery, B.A.

Consultant: J. F. EGGLESTON, B.Sc., M.I.Biol.
Formerly Senior Science Master, Hinckley Grammar School

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Consultant's Foreword

In preparing this book we have been able to select from illustrations that have appeared in the magazine *Understanding Science*. These provide lucid, attractive visual communication which we think appropriate at this level. In addition there are many new illustrations. Where possible, subjects are introduced by, or concluded by, practical and theoretical problem-solving material which provides opportunities for teachers to develop in their pupils an investigatory approach to the subject.

We have attempted to stimulate readers by the challenge of curiosity. Almost all the problem-solving material has been used with classes within the C.S.E. band of the ability spectrum, and their favourable reaction was the main criterion for its inclusion.

C.S.E. Biology is seen by the majority of the Regional Boards not merely as the acquisition of knowledge, but as an opportunity for the use of practical and cognitive skills. The expectation is that assessment procedures which attempt to measure these abilities will have a beneficial 'backwash' effect; they will ensure that teachers whose concern is the development of these skills will be encouraged rather than inhibited by the way in which their product is measured.

One hopes that at least a start has been made on a generation of text books which incorporate this aim.

J. F. Eggleston.

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The first volume in this series of biology books, 1. Common Core, covered the basic biology requirements of the various C.S.E. syllabuses. This, the fourth volume, deals with an *optional* part of the syllabus together with its companion volumes, 2. Genetics and Evolution and 3. Ecology and Applied Biology.

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1. A practical introduction to bacteriology

A practical introduction to bacteriology

Many people find the study of bacteria an especially fascinating part of biology. By no means the least important reason for this interest is the very direct part bacteria play in our lives. Apart from their activities in causing diseases and spoiling food, bacteria are involved in maintaining the fertility of the soil on which we rely for food. We also make use of bacteria in various food-making processes and in purifying sewage. The preservation of foods, the treatment of water supplies, and the prevention and treatment of certain illnesses all require a knowledge of bacteria and their activities. The story of the discovery of bacteria, which is quite recent history, is important because it shows how man learned to perform experiments. It is fitting, therefore, that we should begin our studies of bacteria with a practical introduction. A suitable topic – one of many we might have chosen – is the problem of the souring of milk.

A NOTE ON THE BIOLOGY OF MILK

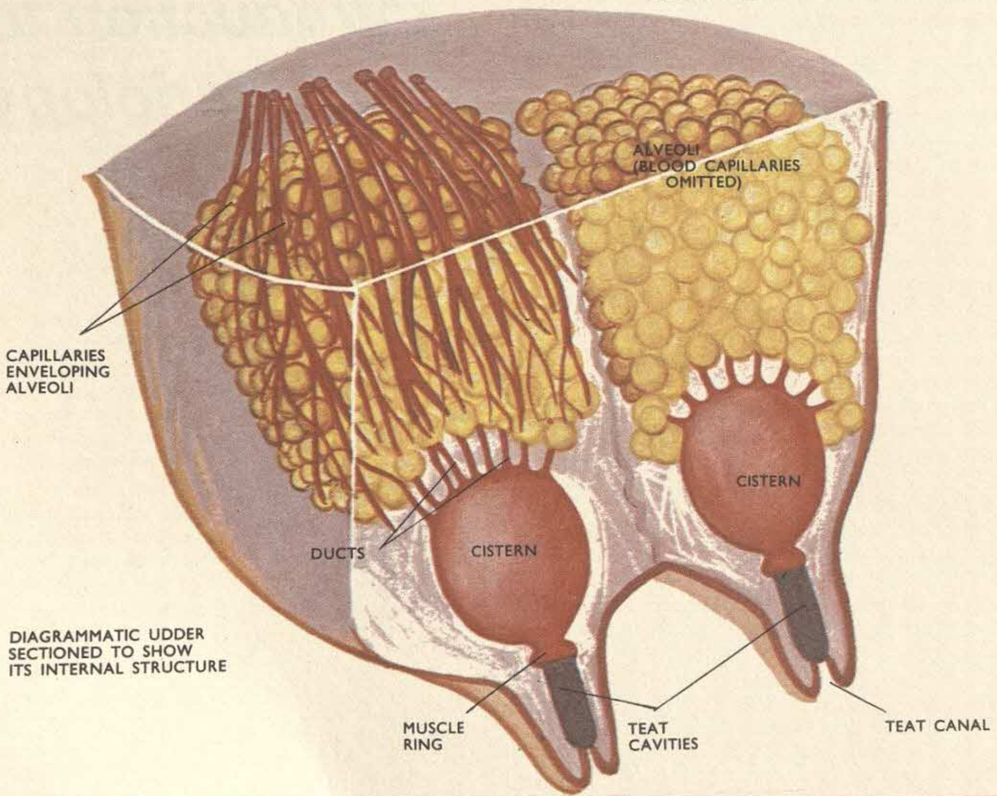
The mammary gland

A cow's udder consists mainly of clusters of gland cells grouped to form numerous tiny, hollow sacs or alveoli. *Each alveolus is surrounded by blood capillaries from which the gland cells obtain their nourishment and the substances they convert into milk. In the lactating animal, milk is poured continuously into the hollow centre of each alveolus and leaves by a duct. The ducts of the many alveoli join like the tributaries of a river and empty their contents into the storage chamber or* cistern *above each teat. It is thought that the mammary glands may be modified sweat glands because of certain similarities in structure.*

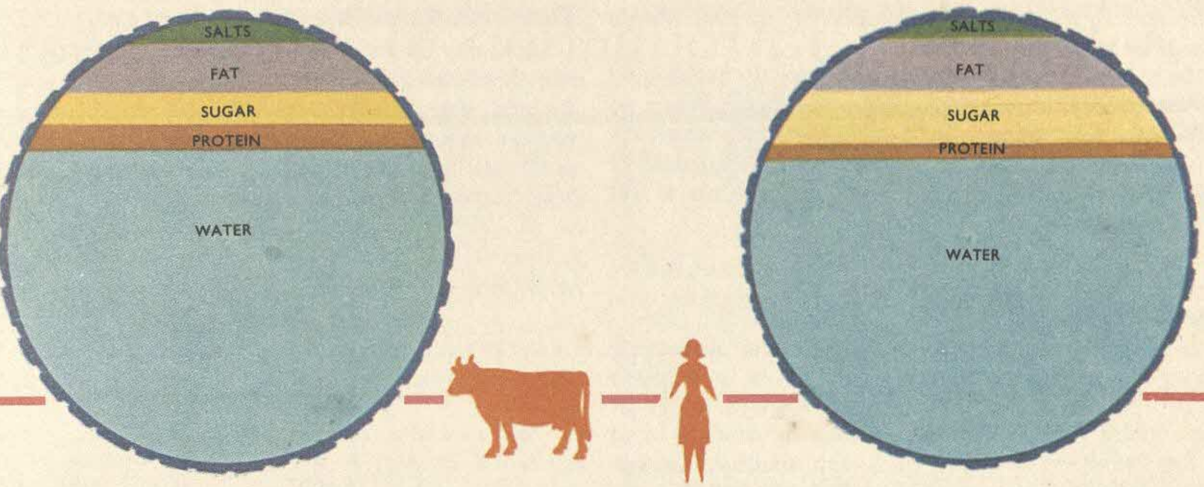
What is milk?

Milk is the fluid produced by the mammary glands, after the birth of the young mammal. It is a fluid characteristic of mammals; these are the only animals to suckle their young.

Milk is composed principally of water – around eighty-seven per cent – and also contains proteins, milk sugar or lactose, most vitamins – even though the quantities of some are negligible traces – fat and various salts. It is particularly rich in calcium, which is of obvious importance to the growing young animal whose sole source of food initially is milk. Calcium is required in large quantities for bone formation. The amount of calcium in human milk is, not surprisingly, much lower than that in cow's milk. The demands of a growing calf are way beyond those of a much slower growing and smaller human youngster. The percentage of protein in cow's milk is correspondingly higher.


ALVEOLI (BLOOD CAPILLARIES OMITTED)
CAPILLARIES ENVELOPING ALVEOLI
CISTERN
DUCTS
CISTERN
MUSCLE RING
TEAT CAVITIES
TEAT CANAL

DIAGRAMMATIC UDDER SECTIONED TO SHOW ITS INTERNAL STRUCTURE



Diagrams showing the major constituents of cow's milk (*left*) *and human milk* (*right*). *The principal constituent is water. Note that the percentage of protein in cow's milk is higher than in human milk as is the salt proportion, the latter being largely due to the large quantities of calcium present. The growth rate of a calf is much greater than that of a child, hence the greater requirement for protein* (*muscles*) *and calcium* (*bones*).

Milk is a type of colloid known as an emulsion (a three to four per cent emulsion of fat stabilized by milk protein); that is, physically it consists of a liquid dispersed within a liquid. The slightly yellow tint of the cream is due to the pigment *carotene*, a precursor of vitamin A. A layer of cream forms on top of the more watery remainder because the larger fat droplets float to the surface. Milk is usually slightly acid, having a pH of between 6.6 and 6.8.

The main protein of milk is *casein*. About three and a half per cent of milk is protein: two-thirds of this is casein.

Analysis of the casein molecules shows it to contain all the essential amino acids that the body is unable to make itself.

The only sugar present in milk is *lactose*. It is found in the milk of all mammals and is formed in the mammary gland and no other part of the body. Just under five per cent of lactose is usually present in cow's milk, but human milk contains up to seven per cent and a cow elephant as much as seven and a half per cent. In a doe rabbit only two per cent lactose is found. Thus the composition and quality of milk of different species of mammals varies considerably. It is interesting to note that in whales' milk there is twelve times more milk-fat and four times more protein than there is in cow's milk. Fat is of great importance as a heat insulatory material to aquatic mammals and also as an energy store for their long migrations from polar seas to more temperate waters.

The main salts present in milk are the chlorides and phosphates of calcium, sodium and potassium. Calcium and phosphorus are particularly necessary for teeth and bone formation. Each litre of milk contains 1,200 milligrams of calcium (cheese, incidentally, contains nearly that amount in each 100 grams).

The formation of milk

The udder of a cow has a rich blood supply and prominent milk veins can be seen on the abdomen of a dairy cow. It might be expected that the milk-producing cells of the udder merely filter substances from the blood, thus making milk. But this is only partly the case. Certainly blood contains sugars, fats, proteins, vitamins, salts and all the other ingredients of milk. However, the sugar in the blood is glucose: that of milk is lactose. Obviously, therefore, chemical changes occur in the udder during the formation of milk.

Most of our knowledge of some of these has been derived from the use of isotopic tracers. For example, labelling of glucose with carbon-14 – a radioactive isotope of carbon – has shown conclusively that milk sugar (lactose) is formed from glucose derived from the blood.

Similar experiments 'labelling' fat in the blood have shown that some of the milk fat is derived from blood fat without chemical change.

The actual quality of milk produced depends to a certain extent on the cow's diet, though the latter can vary enormously without effect. A general deficiency of food lowers the volume of milk produced but not the quality since the cow's reserves are drawn upon to make up for dietary deficiencies. An example of this is the drain on calcium reserves. A diet continuously low in calcium results in a great loss of calcium from the bones, in acute cases even producing skeletal abnormalities which may interfere with the calf-bearing abilities of the cow. The butter-fat content of milk may vary, however, with the diet. A cow fed on young grass or on a diet low in roughage but high in concentrates may show a butter fat depreciation of more than fifty per cent.

The first question to be considered is *what changes take place when milk goes sour?*

As in all investigations, we must use the techniques at our disposal in order to *observe* and *describe*. When we have done this, questions will become clear to which the answers will be sought by inventing explanations (hypotheses) and then performing experiments to test these hypotheses.

A. Changes of acidity

A common method for indicating and measuring changes in the acidity or alkalinity of a solution is by the use of substances which change colour at some point on the scale which may be thought of as a line between very acid and very alkaline, marked off in units of acidity. Litmus and phenolphthalein are examples of such indicators.

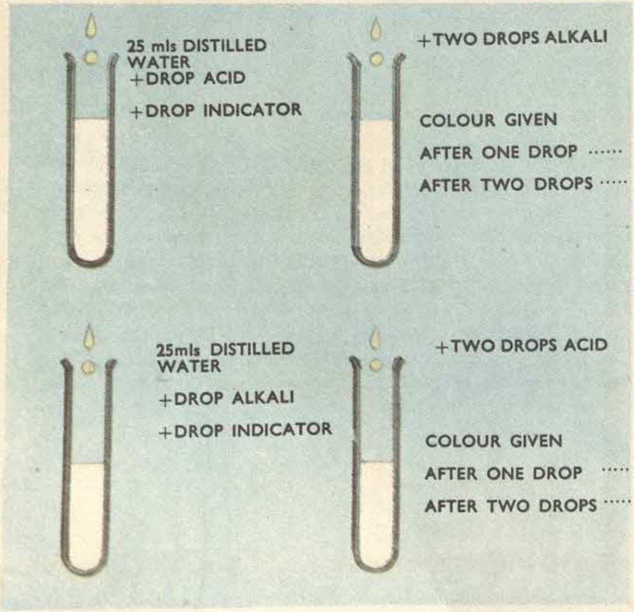
B. Preliminary experience in using indicators

(i) Add a drop of bench (2N) hydrochloric acid to 25 ml. distilled water. Now add a drop of phenolphthalein. Observe and retain.

(ii) Add a drop of bench sodium hydroxide (2N) solution to 25 ml. distilled water. Now add a drop of phenolphthalein. Observe and retain.

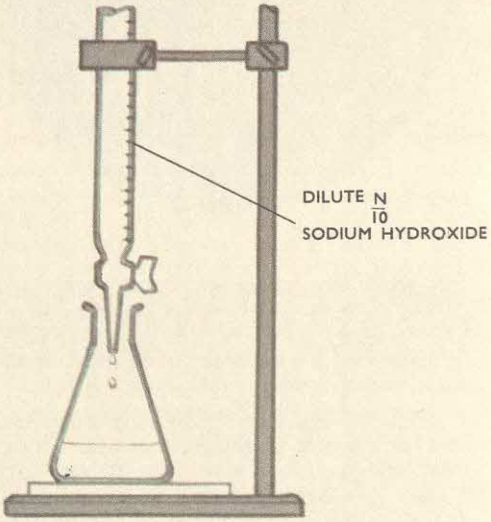
(iii) Now add one drop and then a second drop of bench sodium hydroxide to (i) above and observe.

(iv) Add one drop and then a second drop of bench hydrochloric acid to (ii) above and observe.



C. Acids in milk

One way of measuring the quantity of acid in a sample of milk is to find out how much alkali is required to neutralise any acids present. To do this you will require a burette, a clean conical flask and white tile, a 25 ml. pipette, phenolphthalein and some dilute (N/10) sodium hydroxide solution.



Pipette 25 ml. of fresh milk into a white basin and add one drop of phenolphthalein. Rinse the burette out with distilled water and then with N/10 sodium hydroxide, allowing the latter to run through the burette tap. Fill the burette to the 0 mark on the scale with sodium hydroxide solution.

Now run the sodium hydroxide solution into the milk one drop at a time until the milk *just* turns pink. Repeat this measurement.

Repeat this exercise with samples of milk which have been kept in the laboratory in open test-tubes for one, two and three days.

What do your results suggest about the acid content of milk which has been exposed to air?

Table of results:

	Mls of N/10 Sodium Hydroxide required to neutralize 25 ml of milk					
Dates						
Sample	1	2	3	4	5	6

D. Changes in the oxygen content of milk on exposure to air

Under certain special conditions (the exact nature of which will be discussed later) the 'oxygen content' of a sample of milk can be estimated by the addition of a standard solution of a substance called resazurin. When resazurin is added to milk the mixture goes blue. When the mixture is warmed to 40°C. in an air-tight tube the mixture may remain blue or change colour to pink or white. The results may be interpreted thus:

Mixture remains blue = high oxygen concentration
Mixture goes pink or white = low oxygen concentration.

When performing this test it is very necessary to make sure that nothing which is likely to lower the oxygen concentration in the milk under test can come into contact with the apparatus or chemicals which are used. Certain precautions are therefore taken. These may seem a little tedious, but they are important.

Stage (i). To make up the resazurin solution

Resazurin is purchased from suppliers of biological chemicals in tablet form. The usual strength at which these are produced, e.g. by the British Drug Houses Ltd., is such that one tablet is dissolved in 10 ml. of distilled water to obtain working-strength solution.

The glassware used in making up this solution must be placed in a pressure cooker for 15 minutes at 15 lb./sq. inch. The distilled water must be recently distilled.

Stage (ii). The resazurin test

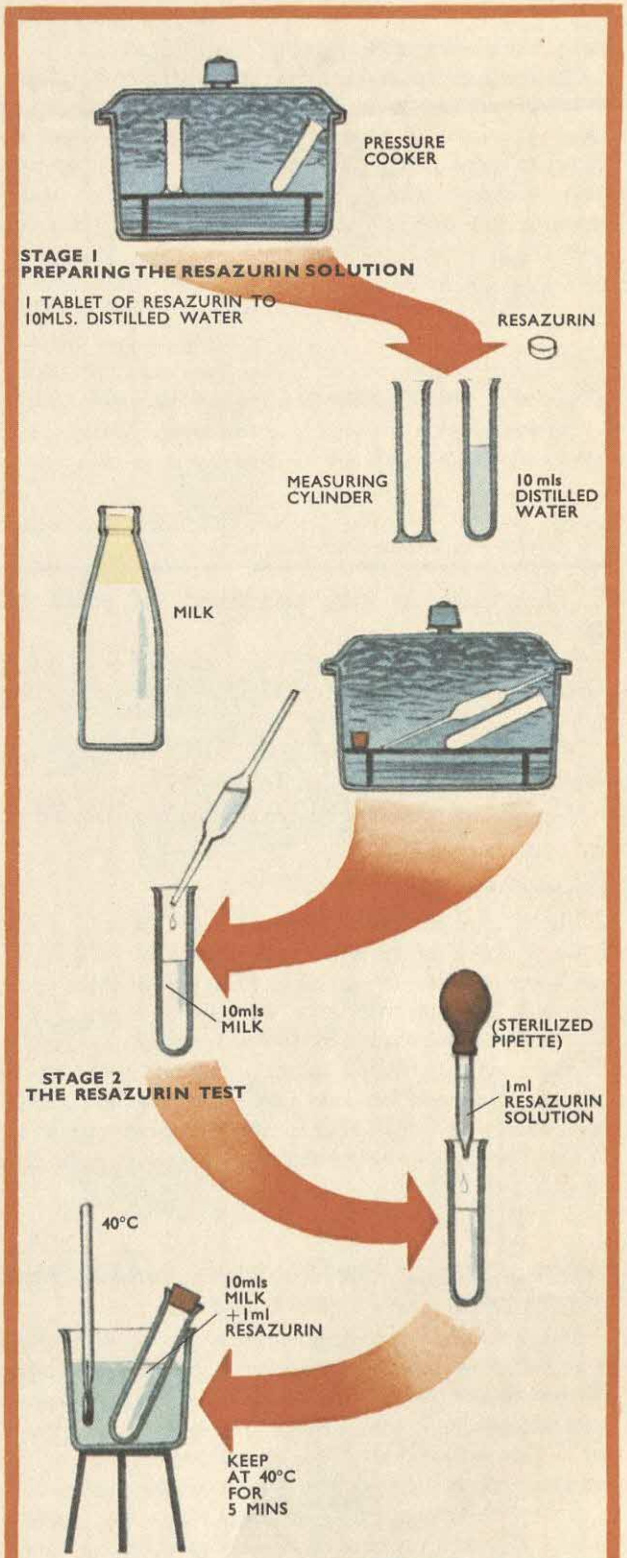
One ml. of resazurin solution is transferred to a tube containing a 10 ml. sample of milk under test. The tube is sealed with a rubber bung. The pipette used to transfer the resazurin, the tube used to contain the milk sample, and the bung must all have been placed in a pressure cooker for 15 minutes at 15 lb./sq. in.

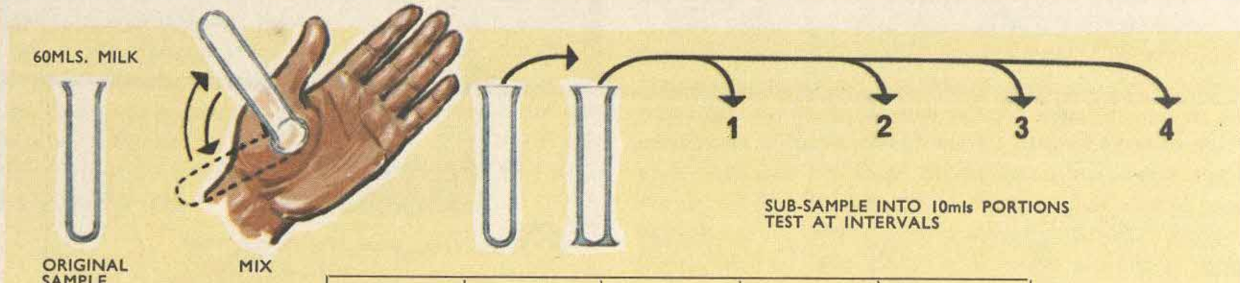
The resazurin and milk sample are now mixed by inverting the tube carefully and the tube is placed in a hot-water bath at 40°C. The level of water in the water bath should be in line with the level of milk in the tube.

During the next five minutes note any colour change and the time taken for any such changes to occur.

The oxygen content of milk at different times can be indicated by the resazurin test as follows:

Samples of milk taken from the same bottle of well-mixed fresh milk are allowed to stand open to the air at room temperature. Samples are tested at once and after two, four and six days. The results are recorded and compared.





TABULATE RESULTS,

Day	0	2	4	6
RESAZURIN TEST COLOUR				
RELATIVE OXYGEN CONCENTRATION				

E. Changes in the reaction of milk to boiling

Apart from the characteristic smell of sour milk, perhaps the commonest test applied is that of boiling the milk and observing the result.

Boil 20 ml. samples of fresh, two-, four- and six-day-old* milk and describe the results.

* i.e. milk which has been exposed to air at room temperature.

Summary

These chemical and physical tests have revealed some of the changes which take place in milk which has been exposed to air, after its removal from cows. The milk becomes more acid, the oxygen concentration of the milk decreases and the reaction to boiling changes with increasing time. We are in a position to ask some obvious but not easy questions. Where has the acid come from? Where has the oxygen gone to? What determines the readiness with which milk clots on boiling?

What causes the changes which take place when milk goes sour?

In the souring-of-milk problem there is not one but a number of events – increasing acidity, decreasing oxygen concentration etc. – each of which may have a separate cause. It may help to simplify the problem if we at first *assume* that all the events are connected. We refer collectively to these events as 'going sour'.

The problem recognises milk in two states, namely fresh and sour. We suppose that something happened to fresh milk to make it sour. Milk is manufactured and stored in the milk (mammary) glands of cows. Once milk is removed from the cow it may go sour. This might be accounted for by one of three explanations:

(i) The milk may contain something which is chemically unstable or which, for some other reason, results in the inevitable conversion of part of the milk into substances not originally present, e.g. acids or substances which absorb oxygen.

CAUSE

Before this problem is considered it will be useful to think about the word cause used in the question. Scientists observe events and search for causes. If a football is observed moving through the air at a certain speed in a certain direction, we may ask what has caused this event. From our past experience we may reasonably expect that the observed event was preceded by another event, for example a boot striking the ball with a certain force at a particular angle. It is unthinkable to us in the twentieth century that a ball at rest would suddenly, without cause, fly through the air in the way described. We are so used to thinking of events being preceded by causes that it seems unnecessary to point this out. Yet man has not always thought like this. Even nowadays, events come to the notice of scientists which appear to have no cause which we can identify. When a lump of radio-active material decays it does so gradually, an atom at a time, but what decides that this atom, rather than that, gives up a particle next?

It is sometimes easy to fall into the trap of thinking that early scientists made some very elementary blunders or seemed 'a bit dim' when deciding what their experimental results meant.

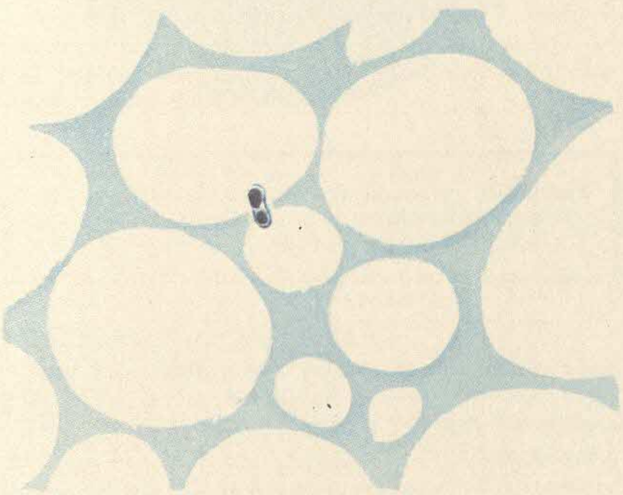
It took mankind a long time to realise that a technique as simple as what we call an experiment could lead to such important, firm conclusions.

(ii) When milk and air come into contact, chemical changes start which result in the changes we call souring.

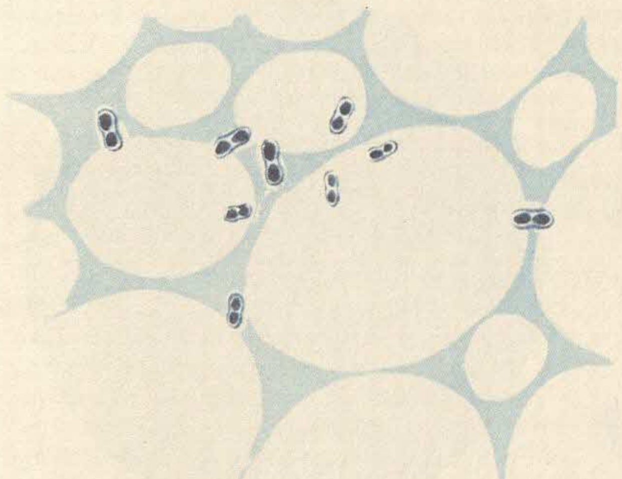
(iii) Something other than air comes into contact with the milk after removal from the cow and causes souring.

F. An observation

Before proceeding with this enquiry make one further observation on milk. This time a microscope is used instead of chemical tests.



(Above) Milk smear made from two-days-old milk – stained with Newman's stain – as seen under a magnification × 900. (Below) Milk smear made in the same way – but the milk was four days old.

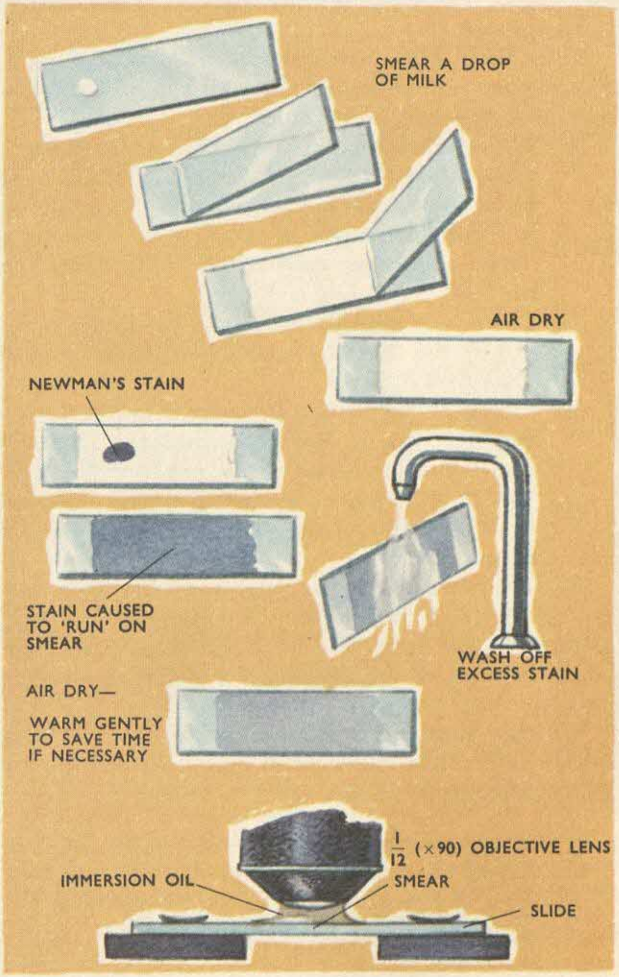


Stained milk smears

Place a small drop of milk on a glass slide. Now touch the slide with the edge of a cover glass which is inclined at an angle of about 45° to the plane of the slide. Bring the cover glass into contact with the milk drop. Wait a second or two until the milk 'runs' by capillarity along the line of contact. Now move the cover glass steadily along the slide so as to smear the milk in a thin layer over part of the surface of the slide. The film should now be air dried by simply waving it about.

Stain the smear as follows:

(a) Add one drop of Newman's stain to smear.
(b) Allow the stain to evaporate in air. *Do not warm.*
(c) Wash off excess stain with running tap-water.
(d) Air dry the smear. At this stage gentle heat can be applied.
(e) Observe under high power and then under oil immersion. (Oil can be applied directly to *dry* smear.)



NEWMAN'S STAIN

To make 100 mls.:

1 gm. methylene blue powder
54 ml. 95% absolute alcohol
40 ml. tetrachloroethane
6 ml. glacial acetic acid

Preparation

Add alcohol to the tetrachloroethane and warm to 70°C. Add solution to methylene blue. Shake well until dissolved. Filter and store in a tightly stoppered bottle.

G. The connection between bacteria and the process of souring

The objects present in sour milk are of interest in our enquiry. They have been seen to divide and grow (reproduce). They have been called germs (many things which grow have been given this name – e.g. wheat-germ, meaning wheat-seed embryo), or, more precisely, bacteria. This new fact adds another part to the problem. We may now ask, *what is the connection between bacteria and the process of souring?* Are bacteria the *result* of the souring process or do they *cause* souring to take place?

Refer back to the three alternative explanations of what causes milk to go sour. Bacteria can be fitted into each of these explanations as follows:

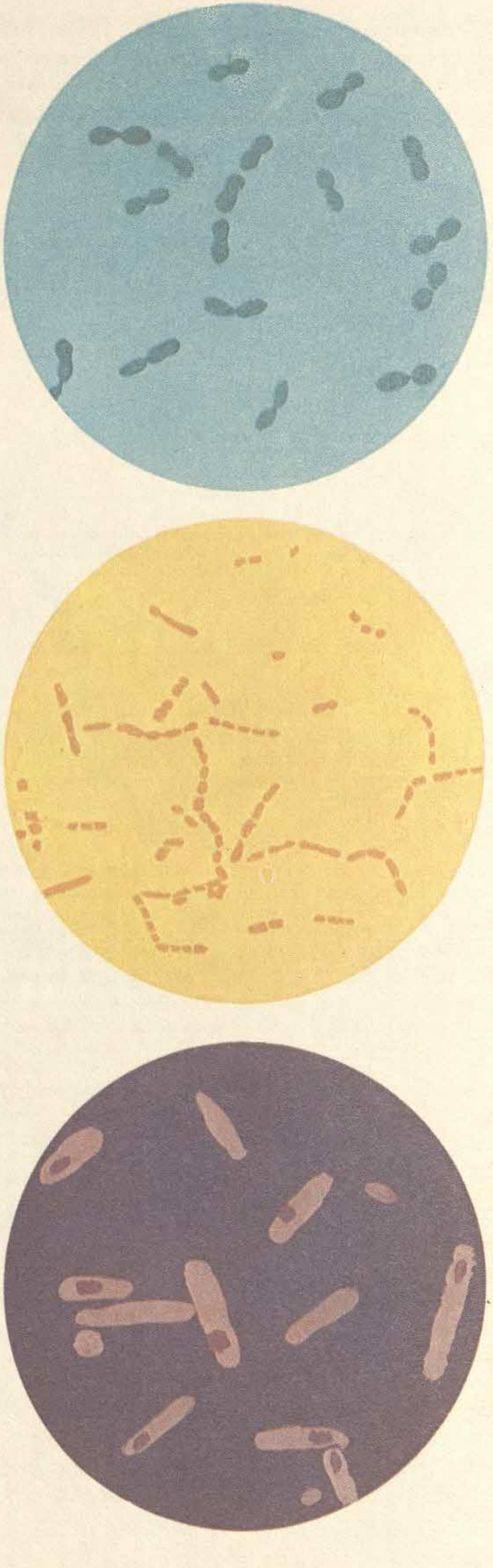
(a) When milk is removed from the cow the changes which take place include the formation of bacteria from milk. Thus fresh milk containing no bacteria becomes sour milk containing bacteria because some of the milk *turned into* bacteria.

(b) The second explanation would be that in order to give rise to bacteria milk must combine with some part of air.

(c) The third explanation is markedly different from the first two. In this case whatever produced bacteria comes from outside, not from the milk. The milk in these circumstances goes sour because whatever 'causes' bacteria is using milk for the process.

At different times in the past scientists have supported one or other of these explanations. A brief history of the development of the argument, with notes on some of the personalities involved, is given later.

We may start our attack on the problem by considering a common observation that *boiled milk does not go sour as rapidly as unboiled milk.*



Some of the bacteria that occur in milk or milk products. From top to bottom: Streptococcus lactis, *an organism that causes milk to sour by producing lactic acid;* Streptococcus thermophilus *and* Lactobacillus bulgaricus, *used in the making of yoghourt; and* Clostridium tyrobutyricum, *an anaerobic bacterium causing blowing of hard cheeses.*

To explain this fact we may use the three explanations (a), (b), or (c) given above. If we favour explanations (a) and (b) we may argue that boiling the milk has interfered with the mechanism of bacteria formation.

The milk takes time to recover from boiling, hence the delay in souring. If, on the other hand, we favour explanation (c) we may argue that whatever causes bacteria (it may be other bacteria) entered the milk from the air before the milk was boiled but was destroyed by high temperature. The delay was due to the time taken for re-infection.

Now perform an experiment which was designed to settle such an argument: boil milk sealed off from air. The principle on which the experiment is based is simple; if the milk later goes sour we must accept the first explanation; if it does not, we must accept the second, or the third.

> Can you think of any ways in which this experiment could be improved? Would a second tube containing milk and open to the air be an adequate CONTROL?

H. The first experiment

Half fill a boiling tube, which has previously been boiled in water in a pressure cooker at 15 lb./sq. in. for 15 minutes, with fresh milk. Test this milk by the test that you decide to use at the end of this experiment. Cover the top of the tube with three or four layers of cooking foil and tie the foil round the neck of the tube with strong thread very securely. Now replace the tube in the pressure cooker for 15 minutes at 15 lb./sq. in.

Set up a second tube which has been treated in the same way as the first, but this time after the addition of fresh milk the tube is neither sealed nor boiled. Also set up a third tube in exactly the same way as the second, except that the milk *is* boiled. All tubes are now set up in the laboratory and after six or more days are tested by any methods to indicate souring, such as acid content, resazurin test – *but not taste.*

Discussion of results

If the untreated milk has gone sour and the treated milk has not, we may be in a position to decide which of the three explanations to accept.

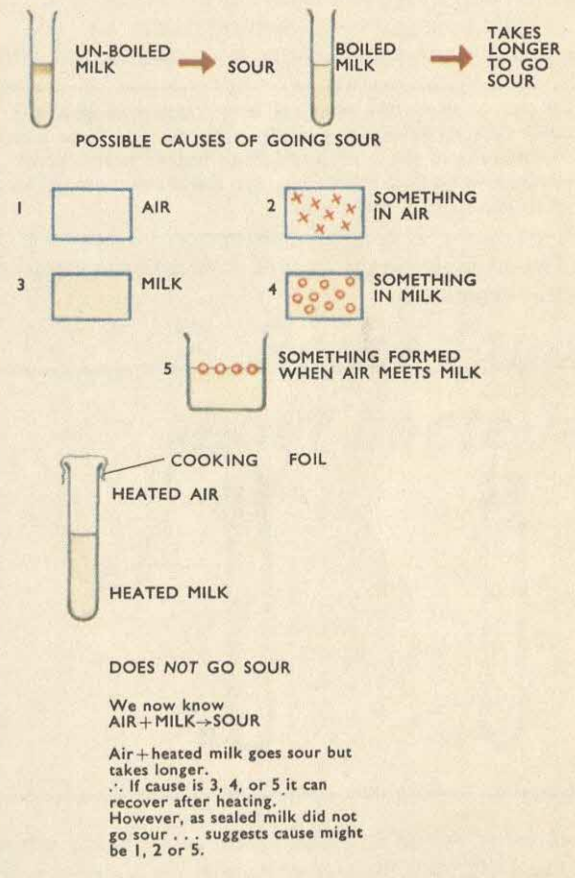
We may describe the three explanations in simple form as follows:

a milk ——→sour milk
b milk + fresh air——→sour milk
c milk + something in air (but not air itself)
——→sour milk

The observation that boiled milk takes longer to go sour than unboiled milk does not help us to distinguish between these three explanations. It is always possible to argue that the milk is affected by boiling in a temporary way which would slow the processes leading to souring in either explanations (a) or (b). Similarly, explanation (c) can be used by asserting that the milk or 'the something' from the air had been affected by heat.

If the results of experiment (H) are as suggested, however, it will be possible to distinguish between explanation (a), which must be wrong, on the one hand and explanations (b) and (c), either of which may be correct, on the other.

What information do the two control tubes supply which is essential to this argument?


UN-BOILED MILK
SOUR
BOILED MILK
TAKES LONGER TO GO SOUR
POSSIBLE CAUSES OF GOING SOUR
1
AIR
2
SOMETHING IN AIR
3
MILK
4
SOMETHING IN MILK
5
SOMETHING FORMED WHEN AIR MEETS MILK
COOKING FOIL
HEATED AIR
HEATED MILK
DOES *NOT* GO SOUR
We now know
AIR + MILK → SOUR
Air + heated milk goes sour but takes longer.
∴ If cause is 3, 4, or 5 it can recover after heating.
However, as sealed milk did not go sour . . . suggests cause might be 1, 2 or 5.

This experiment has allowed us to take one step towards a solution of the problem. We now know that unheated 'air' plays a decisive part in the process of souring and hence bacteria formation. What we do not know is whether unheated air plus milk alone are sufficient to cause souring and form bacteria.

I. The second experiment

The practical problem to be overcome, in order to find an answer to this question, may be put as follows. Milk must be boiled and then allowed a free supply of air from which any possible bacteria-forming particles have been removed.

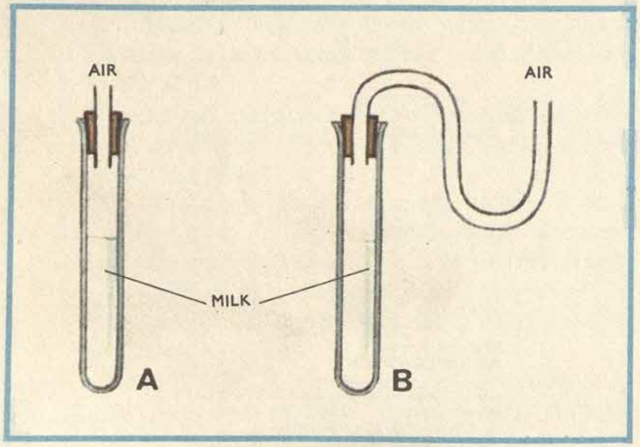
It may surprise the reader to learn that nearly a hundred years elapsed from the time an experiment essentially like the previous experiment (H) was performed, to the time this practical problem was solved to the satisfaction of critics.

Air consists of the gases nitrogen, oxygen and traces of other gases such as carbon dioxide, and the inert gases like argon. Almost invariably solid particles are also suspended in these gases. If it were possible to separate air from the particles it carries it would be possible to distinguish between the alternatives:

milk + air gases ⟶ souring (bacteria)
and milk + airborne particles ⟶ souring (bacteria)

If you examine the furniture in a classroom you will find that there is more chalk dust on the floor and furniture near the blackboard than further away. Chalk dust particles float in air. How can the distribution of the dust particles be explained?

This principle is the basis of the apparatus employed in this experiment.



Two tubes, A and B, contain fresh milk. Test a sample of this milk with the test you apply for souring before you start. Each tube, complete with milk, bung, and glass tubing, is now placed in a pressure cooker for 15 minutes at 15 lb./sq. in. The water in the pressure cooker is now allowed to cool to about 80–90°C. and the tubes are removed. The tubes with the bungs checked for fit are now placed in a shaded part of the laboratory.

If six such pairs of tubes are set up by a class, three may be tested by any test for souring after seven days, the remainder after 14 days by the same test.

Answer the following questions

What happens to any heat-sensitive bacteria-forming 'substances' present in the milk during this experiment?

Do both tubes A and B have access to an unlimited supply of air gases?

Could the same criticism be applied to this experiment as to the first – that the air which comes into contact with the milk may have been altered in some way?

Which part of the experiment is the CONTROL?

What is the only difference between the condition of the air above the milk in each of these tubes?

If B goes sour as well as A, what must be concluded?

If A goes sour but B does not, what must be concluded?

Conclusion

Usually in this experiment the milk in A goes sour in a matter of days, the actual time depending on, among other things, the room temperature; the milk in B does not go sour even after a few weeks.

The simplest explanation which satisfies all the experimental and observed findings in this sequence of practical work is that the particles in the air which cause these effects (souring) and which cause the formation of large numbers of bacteria are themselves bacteria. It may be concluded that airborne bacteria feed on the milk and reproduce.

Discussion

The air-borne particles which caused the changes associated with souring of milk are bacteria. From the observations discussed on previous pages we have learned that bacteria are extremely small, that they can survive in air, and that on contact with milk some bacteria apparently reproduce rapidly. Presumably the bacteria which grow in milk are using the milk for food to supply energy and the materials needed for growth. Some of the chemical changes which take place during souring can be explained if we suppose that bacteria maintain themselves by the same processes as other living things. Thus, the loss of oxygen from souring milk could be accounted for by aerobic respiration (i.e. respiration making the use of oxygen). The increased acidity of souring milk may also be explained if the respiratory processes which lead to the release of energy are similar in bacteria and other organisms. Carbon dioxide produced by respiring animals and plants forms carbonic acid by combining with water. Animals' muscles when active produce lactic acid as an intermediate product of respiration.

Bacteria found in other situations confirm the view that they are living organisms.

2. Some facts about bacteria

Some facts about bacteria

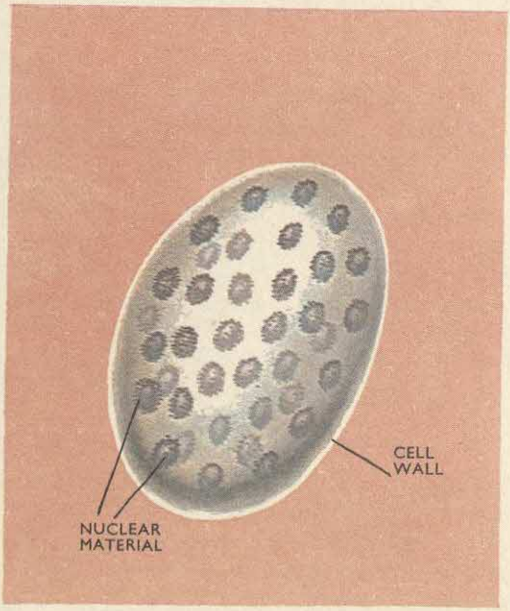
Bacteria are minute, single-celled organisms, so small that they cannot be seen with the naked eye. The largest ones are only about $\frac{1}{1800}$ inch long. The activities of bacteria are essential to life but they may also destroy it. Most are useful, however. For example, some are essential to the processes of decay, helping to break down the dead remains of plants and animals and thus making available materials that plants can absorb through their roots. Others have the ability to use atmospheric nitrogen in the building up of nitrates – mineral salts that are vital for healthy plant growth. Many of the bacteria present in the gut of animals break down food materials and thus make available substances that the animals would otherwise be unable to obtain. Some bacteria are employed in important industrial processes – in the manufacture of acetic acid and cheese, for example.

The most obvious effect of bacterial activity, however, is disease. Many bacteria live on or in the body without ever causing disease, but some may invade the tissues when conditions are suitable. Disease-producing bacteria are called *pathogens*. It is fortunate that only a small proportion of the species of bacteria are pathogenic, but even so the list of diseases that they cause is a lengthy one.

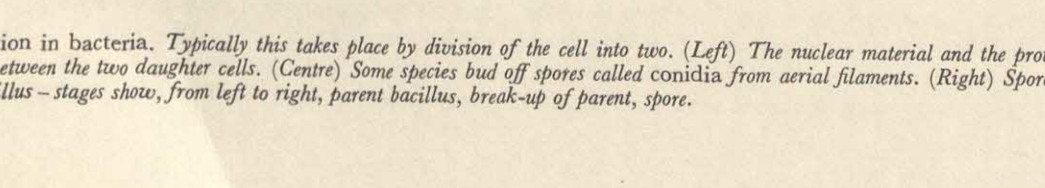
Each bacterium is a living unit that has the ability to manipulate its surroundings chemically in such a way that it can obtain the energy needed to grow and reproduce. The variety of materials that different bacteria use as food is remarkable. Many feed on animals and plants or their products just as we do. Some obtain their energy by oxidizing ammonium compounds, others (the sulphur bacteria) use sunlight (just as green plants do) to form carbohydrates from carbon dioxide and hydrogen sulphide, and the iron bacteria oxidize iron compounds. Yet others can grow and reproduce only inside other living things.

Medical bacteriology is concerned with the bacteria that live in animals, particularly the ones that infect man. Many of these have lost the ability to utilize inorganic substances and have become parasitic, relying on their hosts to provide them with ready-made food. Most of these can reproduce only inside their hosts, though they may be able to survive outside the host by forming a protective coat or *spore*.

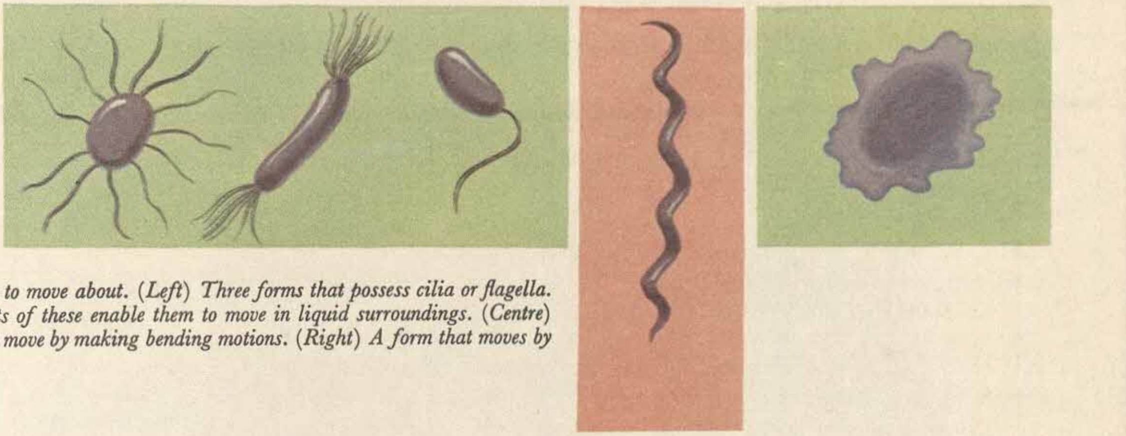
Bacteria may be arranged into three groups according to their shape: rod-shaped forms (*bacilli*), spherical forms (*cocci*), and curved or spiral forms (*spirilli*).



A diagram showing the typical structure of a bacterium. Note the distinct cell wall and the distribution of the nuclear material throughout the cell.



Reproduction in bacteria. *Typically this takes place by division of the cell into two. (Left) The nuclear material and the protoplasm are shared out between the two daughter cells. (Centre) Some species bud off spores called* conidia *from aerial filaments. (Right) Spore formation inside a bacillus – stages show, from left to right, parent bacillus, break-up of parent, spore.*



Some bacteria are able to move about. (Left) Three forms that possess cilia or flagella. The lashing movements of these enable them to move in liquid surroundings. (Centre) A spiral form that can move by making bending motions. (Right) A form that moves by creeping.

Most bacteria have a rigid cell wall. Although they have no nucleus like that of typical plant and animal cells, there is nuclear material scattered through the cell. Bacteria reproduce only by division. Usually the cell elongates prior to splitting across the middle. Sometimes the separation is incomplete and a 'double bacterium' is formed, such as the *diplococcus* that causes pneumonia. Repeated division without complete separation of the daughter cells may produce long chains (e.g. *streptococci* and *streptobacilli*).

Some bacteria are able to move of their own accord, either by means of wriggling movements or by lashing whip-like hairs or *flagella* – this is only possible in moist situations – but the majority are distributed by means of wind, water and animals.

Some bacteria are *aerobic*; that is, they can live only in the presence of free oxygen. Others are *anaerobic*, and can live only in the absence of free oxygen.

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A little history

There is a great deal which man does not yet know about bacteria. Our present knowledge is quite recent – most of it less than a hundred years old. The discovery of bacteria had to wait until the microscope had been invented.

Before bacteria were seen they could only be known by their effects. This history of the investigations which added to man's knowledge of bacteria may be visualised as three strands.

The first strand consists of enquiries into various disease conditions (later some were shown to be caused by bacteria), their prevention and cure.

The second strand was man's attack on the problem of decay and attempts to improve methods of food preservation.

The third, which links the first two as well as including independent studies, followed the invention of the microscope, and consists of the study of bacteria as living organisms, their structure, life history and physiology.

An attempt to illustrate a little of the early history of these investigations, based mainly on the dates of birth and death of the men involved, is shown overleaf. Notice that whereas some scientists' most important findings were in one line of investigation only, others, particularly Pasteur, made major discoveries in all three.

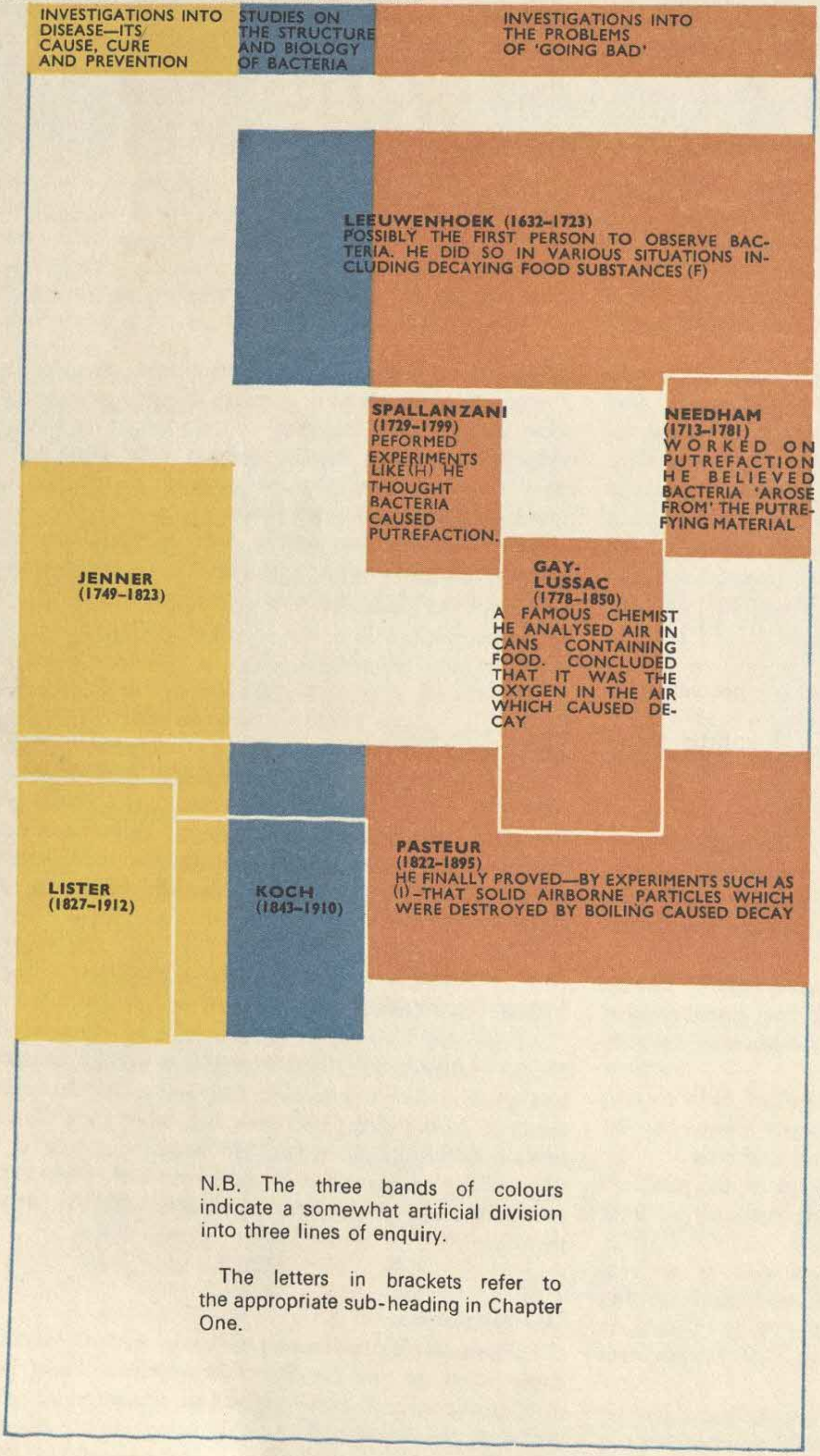
Leeuwenhoek

Anton van Leeuwenhoek was born in the town of Delft, Holland, in 1632. He had a rather unusual background for a scientist, beginning in the cloth trade in Amsterdam, and then becoming City Chamberlain of his native town. He became interested in the making of microscopes, however, and used them enthusiastically in his studies almost until his death in 1723.

The microscopes

Leeuwenhoek's microscopes were not at all like those seen in present-day laboratories. They had only one small lens, almost spherical, mounted between metal plates. Such a lens, although difficult to produce, gave considerably better magnification than

SOME EARLY INVESTIGATORS WHO WORKED ON PROBLEMS ASSOCIATED WITH BACTERIA



LEEUWENHOEK (1632–1723) POSSIBLY THE FIRST PERSON TO OBSERVE BACTERIA. HE DID SO IN VARIOUS SITUATIONS INCLUDING DECAYING FOOD SUBSTANCES (F)

SPALLANZANI (1729–1799) PEFORMED EXPERIMENTS LIKE (H) HE THOUGHT BACTERIA CAUSED PUTREFACTION.

NEEDHAM (1713–1781) WORKED ON PUTREFACTION HE BELIEVED BACTERIA 'AROSE FROM' THE PUTREFYING MATERIAL

GAY-LUSSAC (1778–1850) A FAMOUS CHEMIST HE ANALYSED AIR IN CANS CONTAINING FOOD. CONCLUDED THAT IT WAS THE OXYGEN IN THE AIR WHICH CAUSED DECAY

PASTEUR (1822–1895) HE FINALLY PROVED—BY EXPERIMENTS SUCH AS (I)–THAT SOLID AIRBORNE PARTICLES WHICH WERE DESTROYED BY BOILING CAUSED DECAY

N.B. The three bands of colours indicate a somewhat artificial division into three lines of enquiry.

The letters in brackets refer to the appropriate sub-heading in Chapter One.

1605	Gunpowder Plot
1608	Quebec Founded
1616	Death of Shakespeare
1632	Leeuwenhoek Born
1649	Charles I Executed
1665	Great Plague of London
1702	William III Died
1713	Needham Born
1723	Leeuwenhoek Died
1749	Jenner Born
1766	George II Died
1778	Gay-Lussac Born
1781	Needham Died
1799	Spallanzani Died
1800	Appert devised the process of canning
1822	Pasteur Born
1823	Jenner Died
1827	Lister Born
1843	Koch Born
1850	Gay-Lussac Died
1895	Pasteur Died
1899	Boer War
1910	Koch Died
1912	Lister Died
1939–45	2nd World War
1952	George VI Died

any other microscope of the time. Leeuwenhoek did not invent the microscope (Galileo, often credited with the invention, is known to have used one some fifty years previously). His achievement lay rather in his skill in setting and grinding the lens more accurately than had been possible before.

Blood and circulation

One well-known study by the Dutch scientist was concerned with the circulation of the blood. William Harvey had made the great discovery of the circulation in about 1616, but there remained the problem of how blood from the arteries was actually transferred to the veins to be returned to the heart. Leeuwenhoek's work, together with that of another microscopist, Marcello Malpighi, showed that it passed through tiny tubes we now know as *capillaries*. In other studies Leeuwenhoek examined the structure of the skin, hair, teeth and the eye, observed minute creatures (now called protozoa) in pond water, identified the eggs and pupae of ants and considered a host of other biological subjects.

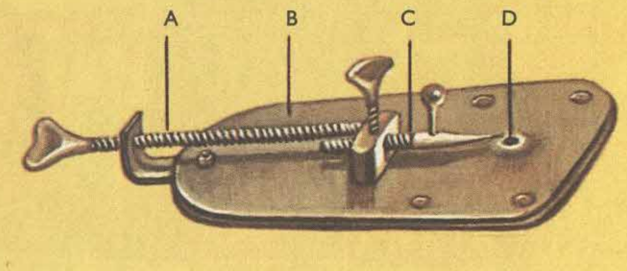
Observation of bacteria

Leeuwenhoek's greatest success came, however, when he examined the tartar from his own teeth. To his great surprise he saw that there were in the tartar 'a large number of "little beasties" moving about in a highly amusing way'. The largest of them, he noted, 'showed the liveliest and most active motion, passing through the saliva as a fish of prey darts through the sea'. This was almost certainly the first observation of the tiny plants called *bacteria* which are so important in our lives as agents of decay and disease.

This Dutch scientist was by no means the only pioneer in the use of the microscope. Robert Hooke, Nehemiah Grew, Marcello Malpighi and Jan Swammerdam were amongst the most famous early workers, opening a vast new field of research into the world of creatures too tiny to see with the naked eye.

Leeuwenhoek shows how to examine a specimen through his microscope.

A Leeuwenhoek Microscope



A. Main adjusting screw for raising and lowering object being examined. B. Microscope body. C. 'Skewer' to impale object and turn it round. D. Simple lens fastened in body of microscope.

Robert Koch – bacteriologist

Louis Pasteur is often credited with being the founder of the science of bacteriology, but Robert Koch, too, must rank as a founder member of this modern and rapidly advancing science.

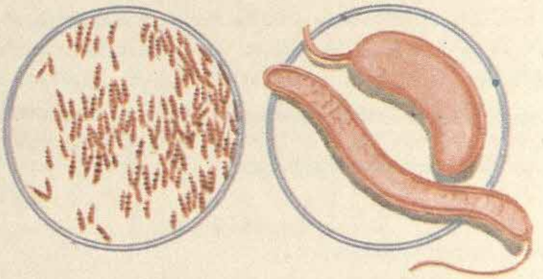
In a period of little more than a decade he and his assistants discovered the causative organisms of no less than eleven diseases.

Koch was born in Clansthal, central Germany, in 1843. After graduating M.D. at Gottingen in 1866 he went into practice, later serving in the Franco-German war as a surgeon. At Wollstein, where he was appointed medical officer in 1872, he began his studies of bacteria.

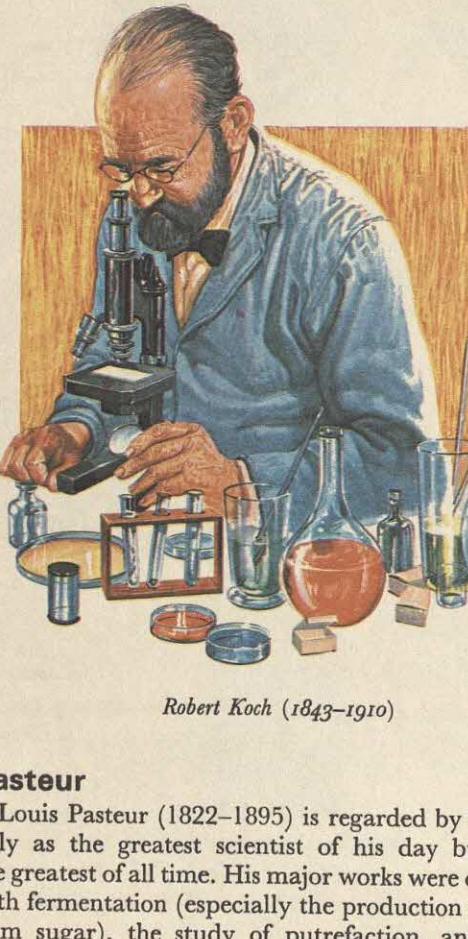
Koch travelled extensively, studying diseases such as cholera, malaria, and sleeping-sickness in Egypt, New Guinea and Uganda respectively.

His first great discovery was made in 1876 when he isolated the anthrax bacillus and showed that it was the cause of anthrax, a contagious disease of sheep and cattle which can also affect man. Perhaps his most important contribution to bacteriology lay in his development of methods of growing or culturing bacteria, particularly that of using solid culture media such as agar-agar jelly. Koch noted that the organisms in his cultures grew into clusters or colonies. After a time these became visible to the naked eye. He realized that the colonies were characteristic for particular organisms.

In 1882, using new staining methods, he discovered the bacillus that causes tuberculosis, a disease in which he showed great interest. A year later he discovered the comma-shaped germs causing cholera, also showing how the disease was transmitted by drinking-water.



The bacilli of tuberculosis (left) and the comma-shaped organisms of cholera (right) which were discovered by Koch.



Robert Koch (1843–1910)

Pasteur

Louis Pasteur (1822–1895) is regarded by many not only as the greatest scientist of his day but one of the greatest of all time. His major works were concerned with fermentation (especially the production of alcohol from sugar), the study of putrefaction, and the investigation of disease-causing micro-organisms. Pasteur brought to all these studies a much clearer understanding of experimental method than any of his predecessors. It is not possible to give an appreciation of his work in this book, but one or two examples will partly illustrate the significance of Pasteur's work.

Consider, for example, the souring-of-milk problem which you experienced. Experiment (H) was in most respects similar to that performed by the Italian scientist Spallanzani. He had heated nineteen 'nutritive broths' (which would certainly go bad if left exposed to air) to boiling point in closed vessels and found that usually putrefaction did not take place. This was one of a number of attempts to demonstrate that solid particles from the air (bacteria or germs) caused putrefaction. Needham, who may be taken to represent opposition to this point of view, believed that putrefaction took place whenever 'nutritive broths' were exposed to air and caused bacteria. Needham did not accept that Spallanzani's experiment had proved his belief wrong. Needham argued that the air above the broth (the vessels were sealed) had been altered by heating. Spallanzani wrote about his work in 1765. Almost a hundred years of argument and inconclusive experiment followed.

During this hundred years the practical application of canning was invented by Appert, and much was learned about the distribution and structure of bacteria.

Louis Pasteur at work in his laboratory using his famous swan-necked flasks.

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It was not until about 1860 that Pasteur produced the experimental evidence which settled the issue.

One such experiment is in principle like (I) in the practical introduction.

It was Pasteur who examined the caterpillars of silk moths which had died from a disease the cause of which was unknown, and showed that bacteria were present. Bacteria were absent from healthy caterpillars and Pasteur demonstrated the infection of healthy moths by bacteria transferred from diseased individuals.

Later he extended his researches to the disease of cattle known as anthrax and to the problem of immunity.

This is but a small part of Pasteur's contribution to scientific knowledge.

Bacteria everywhere

Some of Robert Koch's important contributions to bacteriology were the techniques which he devised for culturing (growing) bacteria. One of these techniques is that of growing bacteria on the surface of a jelly containing food materials on which the bacteria feed. The jelly retains sufficient water to allow rapid growth of bacteria, which usually form circular masses on the surface of the medium. There is nothing mysterious about these 'nutrient media'. The jelly used most commonly nowadays is called *agar* – an extract of seaweed. However, a do-it-yourself culture medium could be made up from gelatin and an Oxo cube!

Nutrient agar may be purchased from suppliers in the form of a jelly or in tablet form. Some agars are specially made up for growing particular species of bacteria, others are such that a much wider variety of bacteria will grow on them. For the purpose of the following exercises one of the latter is required.

Culture methods

If you wish to grow bacteria taken from a particular situation, say 'finger-nail scrapings', it is obviously important to ensure that the bacteria could not have got into the culture from anywhere else.

Consider the steps in the procedure. Nutrient agar must be introduced into a container and then finger-nail scrapings must be placed on the agar.

Now bacteria might be present in:

(a) the agar
(b) the container
(c) the air.

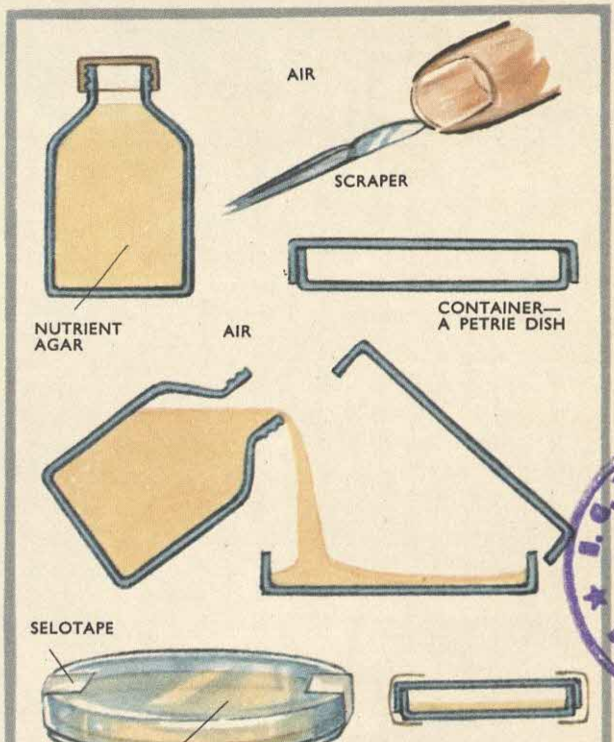
It is therefore necessary to sterilize the agar and the container and to ensure, as far as possible, that the air in the working area is bacteria-free.

The agar should be sterilized in a pressure cooker for 20 minutes at 15 lb./sq. inch pressure.

The petrie dishes, after thorough washing in detergent and later rinsing in distilled water, are air-dried in an oven at 160°C. for 20 minutes.

When the temperature of the oven containing the dishes is down to about 50°C., warm agar – which has been allowed to cool to 50–70°C. in the pressure cooker – is now poured into the dishes. This operation must be done as quickly as possible in an area free from draughts, to avoid contamination by airborne bacteria.

The top is placed on the dish as soon as pouring is complete, and strips of Sellotape are used to secure top on bottom. Whenever a batch of plates is produced in this way they should be kept for a week before use



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to make sure they are sterile. One plate at least should be retained for the duration of the later observations as a control.

Inoculating the plate

The act of adding a sample of material suspected of containing bacteria (in this case finger-nail scrapings) to a medium on which bacteria will grow is called inoculating.

To inoculate an agar plate the lid must be removed and some instrument must be used to transfer the material. Both the air above the dish and the instrument might be infected with bacteria. Again precautions must be taken to minimize the risk of contamination by:

(a) Exposing the plate to air for the minimum time possible.
(b) Sterilizing the instrument to be used and keeping it in sterile conditions until it is used.

Avoid breathing near the plate.

Finally label the plate with a chinagraph pencil (*not* a sticky label) and incubate at 35°C. (or at room temperature if an incubator is not available).

Within a week control and inoculated plates may be observed. The result is usually dramatic and often a little nauseating.

The plates must then be soaked in strong disinfectant.

Further observations

Armed with this basic technique all kinds of interesting investigations are possible. Here are a few examples:

1. Try fingerprints – before and after washing your hands.
2. Try dust from a laboratory window-sill.
3. Cough on an exposed plate.
4. Simply expose a plate to air for 10 minutes.
5. Expose plates to air and use a scent spray to treat half of each plate with different dilutions of disinfectants.
6. Compare plates exposed in well ventilated and badly ventilated rooms, or before and after morning assembly.

The following is an interesting side investigation which, while it does not give very accurate results, is worth trying because the rate of growth and reproduction of bacteria is dramatic. Expose a plate to air and observe daily (always observe through the base of the petrie dish). Select a colony and measure its increase in diameter. Assume the colony to be a single layer of bacteria. If the bacterium producing the colony was circular and $\frac{1}{2000}$ inch in diameter, what can be deduced from these observations?

3. Some methods of food preservation

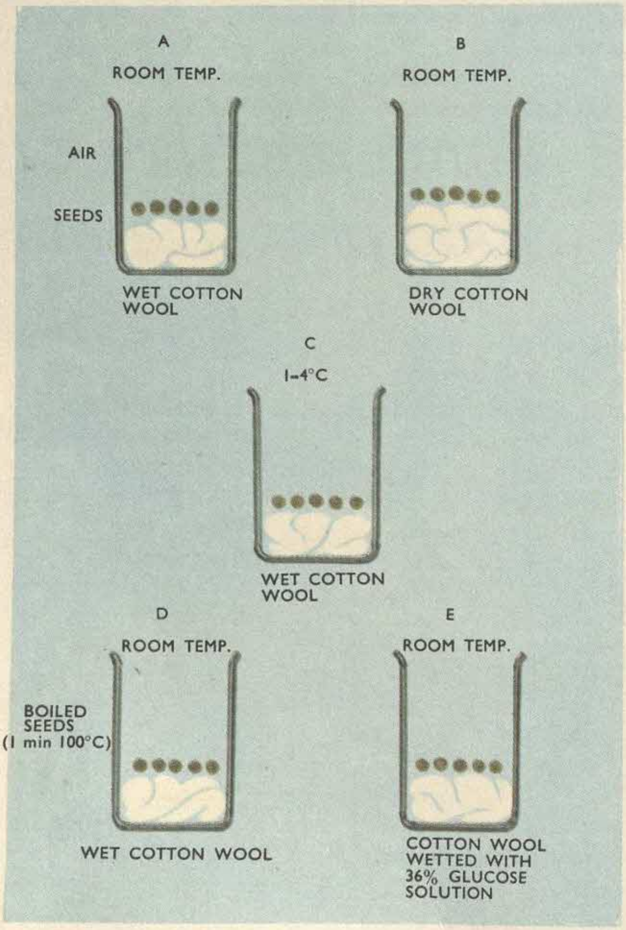
Some methods of food preservation

In a previous experiment we established that if milk was well boiled in a container from which airborne particles were excluded, it did not go sour. However, milk when boiled but exposed to air went sour, although less rapidly than unboiled exposed milk.

This information gives us a technique for food preservation. Providing the organisms (present in the food) which cause decay are killed by prolonged boiling, and re-infection by other organisms from air is prevented, decay does not occur.

This is not the only possible technique of preservation. As we know that bacteria behave in many ways like typical living organisms, it will be useful at this stage to investigate the effects of a number of treatments on living organisms which are a little more familiar and easier to handle – namely cress seeds.

Set up a series of lots of ten cress seeds in 1-inch diameter flat-bottomed specimen tubes as illustrated below.



Examine the tubes after 48 hours. Count the number of seeds showing signs of germination, and for the whole class's results find the percentage of seeds germinating in each condition.

Now return the seeds from tubes B, C and E to the conditions in tube A, i.e. to room temperature with the seeds in contact with water.

Observe after 48 hours for signs of germination.

Answer the following questions

1. The effect of a temperature of between 1–4°C. on cress seeds is:
   (a) To kill them.
   (b) No noticeable effect.
   (c) To speed up their germination rate.
   (d) To slow down their germination rate.
2. In order to be certain of your answer to question (1) it was necessary to know what happened to seeds in:
   (a) Tube B.
   (b) Tubes A and B.
   (c) Tubes A and C (before changing temperature).
   (d) Tube C (before and after changing temperature).
   (e) Tubes A and C (before and after changing temperature).
3. If you wished to keep some seeds for a year in a condition such that they would later germinate, which of the following procedures would you adopt?
   (a) Boil them.
   (b) Keep them dry.
   (c) Keep them in the dark.
   (d) Exclude oxygen from the packet containing them.
4. When dried prunes which have a wrinkled appearance are placed in water they swell and become smooth. If prunes are placed in strong sugar solution they remain wrinkled and do not swell.

How does the interpretation of these observations help explain the results in tube E?

From this experiment a relationship between living processes and temperature can be described. In general, low temperatures just above freezing point slow living processes but do not apparently affect the organism in any other way. High temperatures, on the other hand, if they are over 100°C., apparently damage the living organism so that it can no longer function properly.

The effect of temperature is, however, not quite as simple as this description suggests. Some organisms are killed by temperatures below 100°C. provided they are applied for a sufficient time. Other organisms – especially during resting or spore stages of their life histories – are resistant even to temperatures a little over 100°C.

Living things are also dependent on the presence of water. Growth cannot take place in the absence of water. The removal of water by air-drying or by exosmosis by a concentrated bathing solution prevents seeds and spores developing.

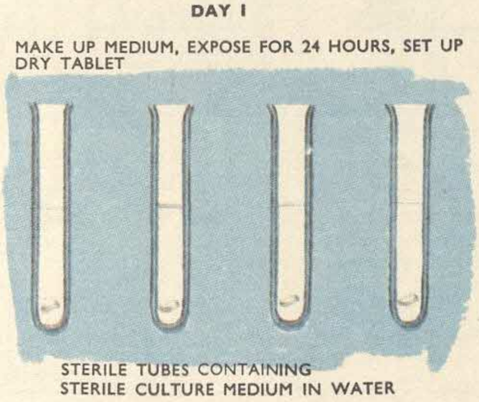
The effect of temperature on the growth of bacteria

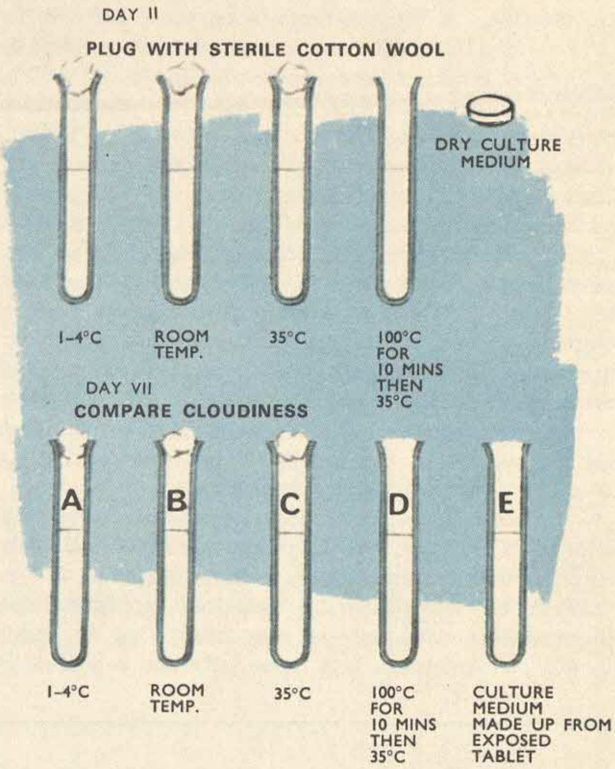
Tablets of solid culture media* can be purchased from biological suppliers. Nutrient broth can be made by adding the tablet to hot water. Four tubes are sterilized by heating dry in an oven at 160°C. for 20 minutes. The directions supplied with the tablets will explain how to prepare sterile solutions of broth.

For the purpose of this experiment the four tubes, once prepared, are left open to the air for one day and are then plugged with sterile cotton-wool. One tube is now placed in a fridge, the second is kept at room temperature, the third at 35°C. If an incubator is not available the third tube could be left near to a radiator. The fourth tube, after exposure to air, is boiled for *ten* minutes, then plugged and left at 35°C. In all cases, direct sunlight on the tubes should be avoided.

The cloudiness which develops in the tubes is a measure of the bacterial growth which has occurred.

Leave a tablet of medium exposed to air for the duration of the experiment. At the end of the time allowed for growth (i.e. from the time the medium was made up to the time the final observations are made) make up a volume of medium equal to that in the other tubes from the exposed tablet.


DAY I
MAKE UP MEDIUM, EXPOSE FOR 24 HOURS, SET UP DRY TABLET
STERILE TUBES CONTAINING STERILE CULTURE MEDIUM IN WATER


DAY II
PLUG WITH STERILE COTTON WOOL
DRY CULTURE MEDIUM
1–4°C
ROOM TEMP.
35°C
100°C FOR 10 MINS THEN 35°C
DAY VII
COMPARE CLOUDINESS
A
B
C
D
E
1–4°C
ROOM TEMP.
35°C
100°C FOR 10 MINS THEN 35°C
CULTURE MEDIUM MADE UP FROM EXPOSED TABLET

Answer the following questions

1. At which temperature is growth greatest?
2. What further information is required in order to find out if refrigeration at 1–4°C. has killed bacteria?
3. In which conditions was growth least? Suggest why.

The processing of milk by pasteurisation

Pasteurisation is a process of applying heat to food and various drinks in order to destroy disease-causing organisms (such as some bacteria) and to reduce the numbers of bacteria and fungi that cause food to decay, thus allowing it to be kept for a longer period of time.

The process is named after the great French scientist Louis Pasteur. It was developed after his work on the souring of wine and beer had shown that heating them to a temperature of about 130°F. (54·4°C.) for a short time delayed souring.

Pasteurisation is a much less severe process than sterilization, the purpose of which is to kill all germs, since it destroys only a small percentage of the food value and hardly affects the flavour of the food. Much higher temperatures are obtained in sterilization than in pasteurisation. Canned meat, vegetables and milk are sterilized at temperatures of between 240–250°F. (115·5–121·1°C.), whereas the temperature at which milk, for example, is pasteurised is only 161°F. (71·7°C.). Eighty per cent of a food's vitamin C content is lost merely by boiling it for 20 minutes, and at 120°C. vitamin B_1 is destroyed. Milk, when it is pasteurised, loses only 10% of its vitamin B_1 and only 20% of its vitamin C.

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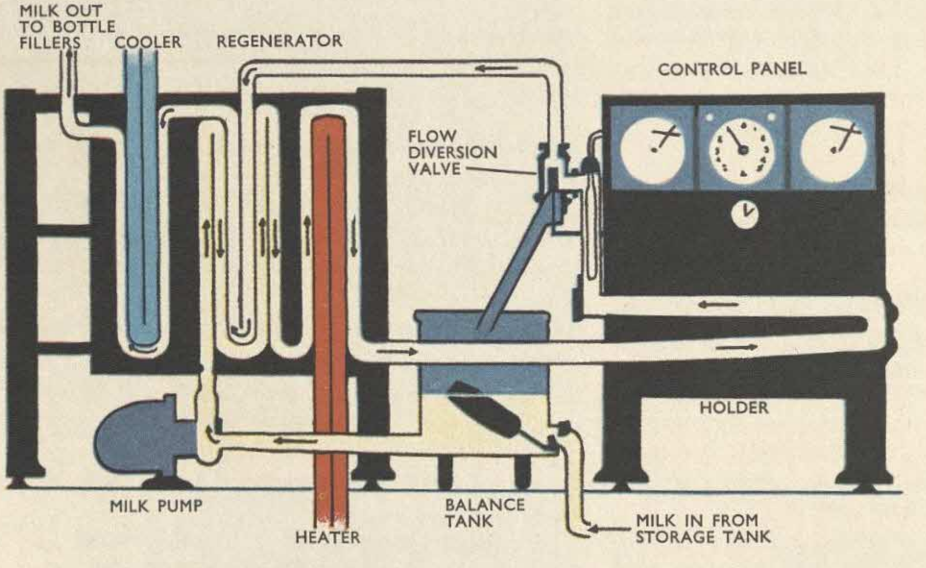
* The media and a handbook of instructions can be obtained from: OXOID Ltd., Southwark Bridge Rd., London S.E.1.

The best-known use of pasteurisation is in the preparation of milk. It consists of heating the milk to 161°F. (71·7°C.), a temperature below its boiling point, for a definite length of time, followed by immediate and rapid cooling. The heat treatment destroys all the disease-causing bacteria which may be in the milk and at the same time reduces the number of bacteria which cause milk to go sour so that its keeping quality is greatly increased.

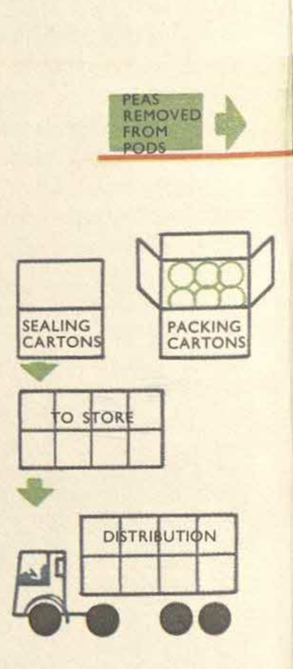
Milk was first pasteurised towards the end of the nineteenth century. Pasteurisation was then used solely to delay the souring of milk. Later, however, it was realised that harmful germs could also be killed if the process were modified. It was found that in order to kill certain germs (e.g. especially the tuberculosis bacillus that causes tuberculosis – a disease of the lungs that caused many deaths in the eighteenth and nineteenth centuries and the early part of the twentieth century) it was necessary to hold the temperature of the milk at approximately 145°F. (62·8°C.) for nearly half an hour. A process using this method is called the *holder* process.

The holder process is uneconomic, however, because it takes too long and too large a plant is required. It has largely been replaced by a higher-temperature, short-time method (H.T.S.T.) in which the milk is retained at a temperature of 161°F. (71·7°C.) for 15 seconds. This is sufficient to kill the disease-causing bacteria, including the tuberculosis bacillus, and most of the bacteria which cause souring. The milk is then cooled rapidly to below 50°F. (10°C.). If the milk were allowed to cool slowly, conditions would encourage the growth again of the milk-souring bacteria and so rapid souring would take place.

As the diagram shows, the cold incoming milk is first heated by the hot outgoing pasteurised milk as the latter passes from the 'holding tube' through the regenerator. The pasteurised milk loses heat to the



A simplified diagram showing the flow of milk through a H.T.S.T. pasteurisation plant. The heater, regenerator and cooler are each made up of a large number of thin, stainless steel plates so arranged in a frame that there is a small gap between each plate and its neighbour. Milk flows in a thin film over one side of the plate and the heating or cooling liquid over the other. In the regenerating section, the treated but still hot milk flows on one side and the incoming cold milk on the other. By this means the former is partially cooled and the latter partially heated by an exchange of heat between the two and a considerable economy results.



cold milk and thus is itself partly cooled. The warmed incoming milk is then heated by hot water in the heater to 161°F. (71·7°C.). Its temperature is carefully maintained so that it enters the holding tube at 161°F. The milk takes only 15 seconds to pass through the holding tube, its temperature remaining at 161°F. during this time. It then passes to the regenerator, where it heats more incoming cold milk (itself being slightly cooled) and is piped to the cooler, in which it is cooled by brine or chilled water to 40–45°F. (4·4–7·2°C.).

A safety device, the flow diversion valve, automatically opens if the temperature of the milk at the end of the holding period is below 161°F. (71·7°C.) and the milk is returned to the heating section. When the required temperature is regained, the valve closes and the pasteurised milk passes to the regenerator.

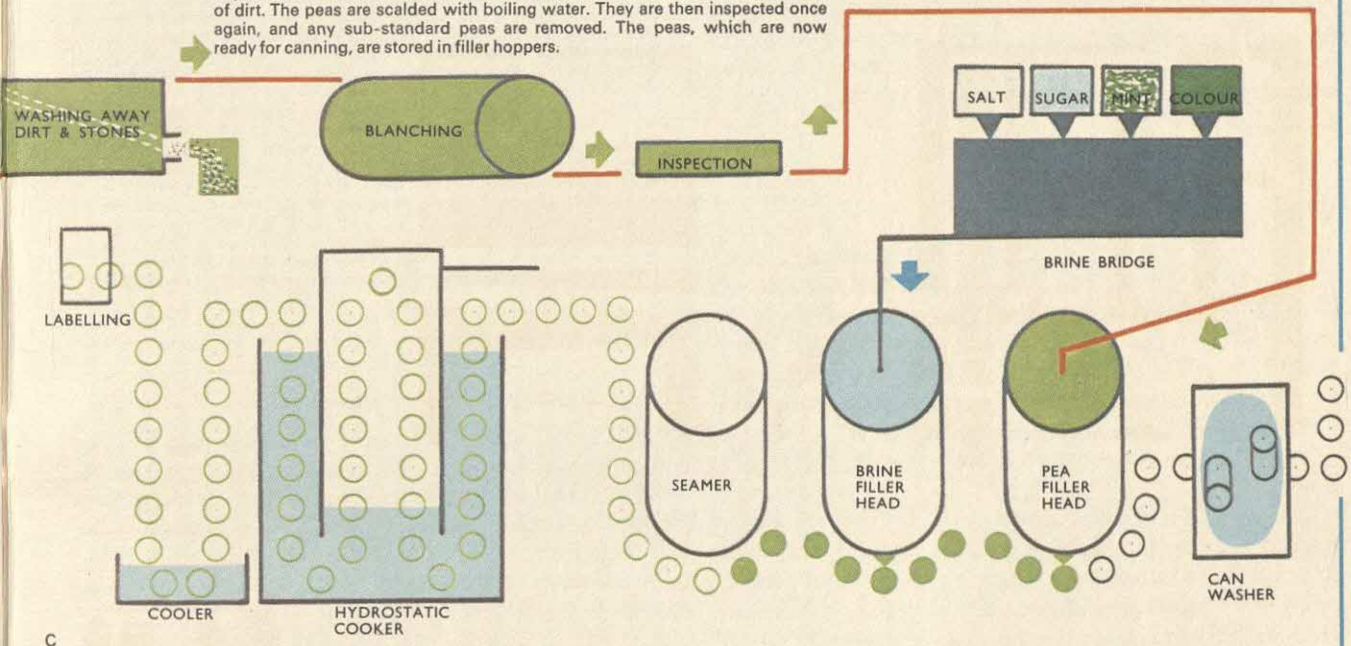
Freshly pasteurised milk is immediately filled into bottles, which have been thoroughly washed and sterilised beforehand to prevent re-infection with bacteria. The bottles are capped automatically with aluminium caps which are cut from a ribbon of aluminium foil.

Canning

Before the beginning of the nineteenth century the choice of foodstuffs for use during the winter months was very much restricted and often resulted in unbalanced diets. Because they deteriorated quite quickly, many fruits and vegetables were available for only a short period after they had been harvested. Some foods, notably meat and fish, were preserved by smoking, salting or pickling, while certain fruits could be dried in the sun. But much of the nourishment, in particular the vitamins, was lost from some of these foodstuffs while they were being preserved.

Since those days various techniques have been developed to protect food against deterioration. The principal methods in use today are refrigeration, dehydration and canning. In consequence, virtually any food which is cooked before it is served can be stored safely for out-of-season use.

Stages in Canning Peas



A The peas which pass the selection test are then *blanched*. This process improves the colour and texture of the peas and also removes the last traces of dirt. The peas are scalded with boiling water. They are then inspected once again, and any sub-standard peas are removed. The peas, which are now ready for canning, are stored in filler hoppers.

C
The peas already sealed in their cans are then sterilized. They are heated by steam under pressure to a temperature of 115°C and remain in the unit for between 20 and 30 minutes. As well as killing off any harmful bacteria which may have been packed with the peas, this also cooks the peas. When there is a large number of cans of one product to be sterilized they are passed through a continuous hydrostatic high-speed sterilizer. However, smaller batches can be handled more conveniently by loading them into enormous pressure cookers called *autoclaves*.

B
Now the lids of the cans are seamed on. This is done by another automatic machine which curls the edges of the lid over the flange of the open end of each can. There should be no air remaining inside the cans.

It was a French chef named Appert who in 1809 laid the foundations of the modern canning industry. Appert recognized the need for vegetables to be fresh and clean when they are preserved, and for the air to be excluded from storage vessels. According to Appert's method, the food was heated for a predetermined time – by experiment he had found the optimum time of heating for different foods – and while the product was still hot it was sealed into a glass jar. Quite soon soldered tinplate canisters – the forerunner of the modern can – replaced the glass jars, which proved to be too fragile.

Preserving

Even in the home, although we are used to handling only small quantities of food, it is a tremendous problem (in hot weather particularly) to prevent the food from spoiling; butter easily becomes rancid, milk quickly sours, meat goes bad, and fruit ripens too quickly. Until the recent introduction of the refrigerator, food had to be eaten very soon after it was purchased from the shops.

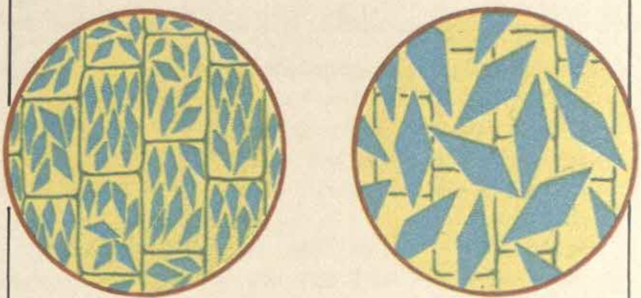
Our problems in this respect may seem considerable, but what of those of the food industry which handles millions of tons of food weekly? Many countries import food from overseas. In Britain about a third of the meat consumed is imported from such far-off countries as New Zealand, Australia, Brazil and Argentina. A journey by ship may take eight weeks, so obviously very special equipment is required to keep the meat in good condition.

Why does food spoil?

There are several reasons for food spoiling. These include: (1) drying up (desiccation) due to loss of moisture by evaporation (this commonly occurs with moist foods); (2) the food may continue to ripen (this happens with fruit); (3) it may become rancid by oxidation in contact with the air; (4) it may become dirty or spoilt by animals such as insects; (5) it may be affected by micro-organisms such as bacteria and fungi.

Many micro-organisms are able to live on foodstuffs, and in doing so they convert the chemical substances of which the food is made into others, causing it to decay. Cheese, for example, can often be seen to have green and blue-green moulds on it. These and the black pin mould commonly grow on moist bread as well. A few bacteria produce poisonous substances called toxins. These toxins cause food poisoning in individuals who eat food containing them.

Quick-freezing



(Left) Small ice crystals are formed in quick-frozen food. (Right) Large crystals are formed when the food is frozen slowly and the cell walls are ruptured.

A decided advance on straightforward cold storage has been the development of 'quick-freezing' as the result of the experiments of the American Clarence Birdseye. Previous to the introduction of this method of preparing foods in 1929, people living in large cities rarely received food in a 'just-picked' condition. But in quick-freezing the food arrives in the home in such a fresh condition.

Birdseye perceived the idea of the 'quick-freeze' treatment of food when he noticed how the Eskimos of Arctic Canada kept food in the intense cold of the open air for several months and yet were able to eat it as fresh when it was thawed. He discovered that providing you freeze a food quickly enough and keep it at a sufficiently low temperature it loses nothing by way of flavour or food value and is still fresh many months later.

When food is frozen slowly, large ice crystals are formed in the cells of the food. The crystals rupture the cell walls as they grow and consequently when the food is thawed the water drains away carrying salts and other minerals with it. So the food loses its flavour and food value. But by the 'quick-freeze' method the ice crystals are formed much more quickly and are much smaller. All the moisture in the food freezes before the crystals have had time to reach a great enough size to rupture the cell walls. When the food is thawed no moisture is lost from it. It thus retains its flavour and its food value and is as fresh after several months' storage as it was at the moment it was frozen.

After cleaning, quality selection and other procedures have finished, the foods are packed into cartons which are placed on metal trays. These are placed in 'frosters' – large insulated cupboards with hollow shelves through which the refrigerant liquid is passed at a temperature of −28°F. (−33.3°C.) (i.e. 60 degrees of frost). The cartons are frozen for an hour to an hour and a half, during which the food at the centre of each carton is cooled to 0°F. (−17.8°C.). From the frosters the cartons, after being packed into boxes, are transferred to large cold stores in which the temperature is sub-zero. They reach the shops either by means of refrigerated containers carried by rail or in refrigerated road vehicles.

The purpose of refrigeration

Food may be kept clean by wrapping it or storing it in suitable containers. Apart from canning or drying food in order to preserve it, the most widely used methods involve refrigeration. By cooling a food below freezing point to 14°F. (−10°C.) all of the bacteria and other minute organisms which cause decay are inactivated; that is, the chemical reactions which normally take place within them cease. Thus decay and the consequent spoiling of the food are delayed. At the same time the enzymes (substances which control chemical reactions in living things) and other complicated chemicals in the food are not destroyed, their activity being merely slowed down.

Refrigeration of Fruit

Like all living structures, fruits age. The processes of ageing carry on even after they have been picked. You can ripen off green tomatoes by leaving them on the window sill in the sun, for example. Fruits will only keep for a very short period if they are not kept in cold storage. A temperature must be maintained in the cold store which is low enough to slow down ripening processes and the activities of bacteria and fungi. However, if fruit is stored at such a low temperature that the sap freezes, the fruit is killed. Even at a less cold temperature it may be damaged chemically and so may fail to ripen, ripen too quickly and go rotten, or be very prone to attack by fungi.

Grapefruit, oranges and tomatoes are stored at 42°F (7·2°C). Bananas need a higher temperature, between 53°F (11·7°C) and 58°F (14·4°C), and temperatures for apples range from 31°F to 40°F (—0·6°C to 4·4°C).

Meat

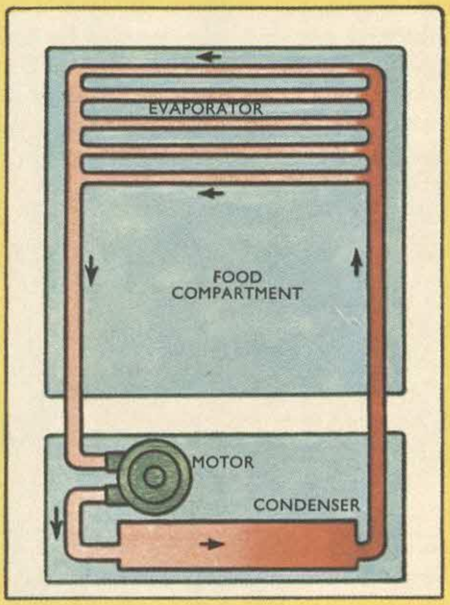
The fact that the activity of bacteria is stopped at low temperatures is of great importance. It means, for example, that in Britain meat can be eaten which has taken several weeks to arrive from overseas and which has been in cold storage here for as long as three months. New Zealand lamb, for example, is cooled to about 8°F (—13·3°C) in New Zealand before it is packed into the refrigerated holds of meat-carrying vessels in which the temperature is held at 8 to 10°F (—13·3°C to —12·2°C). The fastest vessels take four weeks to reach Britain (the slowest about two months) and the meat is then transferred to cold storage warehouses where a temperature of about 14°F (—10°C) is maintained. Very little flavour is lost during three months' cold storage but the meat must be used by the end of this period. If stored for more than three months, flavour and tenderness are lost and the meat starts to deteriorate due to loss of moisture from the surface of the meat (this is called surface dehydration). The atmosphere in the cold storage room is dryer and colder than the meat so that some moisture tends to pass out of the meat into the atmosphere of the cold storage room until eventually the amount of moisture in both the meat and the atmosphere of the cold store would balance if the process were allowed to go on for too long.

Large carcasses lose relatively less moisture than small ones because the surface area of a large object is relatively smaller than that of a smaller object. For this reason beef is easier to keep than lamb even though beef is shipped and stored in quarter carcasses and lamb in whole carcasses.

To transport meat from cold storage to the butcher specially insulated lorries are used on journeys of less than twelve hours. They have no refrigeration plant on board but may be fitted with hollow plates which contain a cold liquid that is frozen before the vehicle begins its journey.

For some kinds of food, such as wet fish, a damp atmosphere is maintained in cold stores. Poultry, on the other hand, is generally wet and warm when it arrives at the cold store. Crisp, cold air is circulated so that excess moisture and heat are removed.

Food kept in cold storage for long periods slowly becomes frozen, and due to the dripping of moisture from it when it thaws and sometimes when it is in the cold store it loses some of its flavour and its food value.



How a Refrigerator Works

When a liquid evaporates (i.e. changes to gas) it takes in heat. In a refrigerator the heat is taken from the food compartment which is therefore cooled. When the gas changes back to a liquid in the condenser heat is given out. This does not raise the temperature of the cold storage compartment because the condenser is always situated outside it.

Accelerated freeze drying

The mushrooms and chicken in a packet of powdered soup look shrivelled and dried up and feel extremely light. This is because they have been treated in an accelerated freeze dryer to prevent them from going bad. Nearly all the water has been taken out of the food and, as food consists largely of water together with a small percentage of proteins, carbohydrates, etc., only a fraction of the original weight is left. The chicken and mushrooms are first of all deep-frozen and then the water is removed in a vacuum compartment.

Accelerated freeze drying is by no means confined to chicken and mushrooms. Almost any food can be preserved this way. It is possible to buy packets of freeze-dried beans, prawns, and even instant dinners.

Some beef and vegetable stew that has been dehydrated after cooking will lose five-sixths of its weight, but will regain this when it is soaked in water again. Until it comes in contact with water it will not go bad.

Bacteria are largely responsible for the deterioration of food. They live on the food and multiply, causing its decay. It is almost impossible to keep fresh food from becoming contaminated by them. But the bacteria can

Storage

Food that has been preserved by accelerated freeze drying does not need storing in a refrigerator. All the water has been removed from the food and therefore there is nothing left it to be frozen.

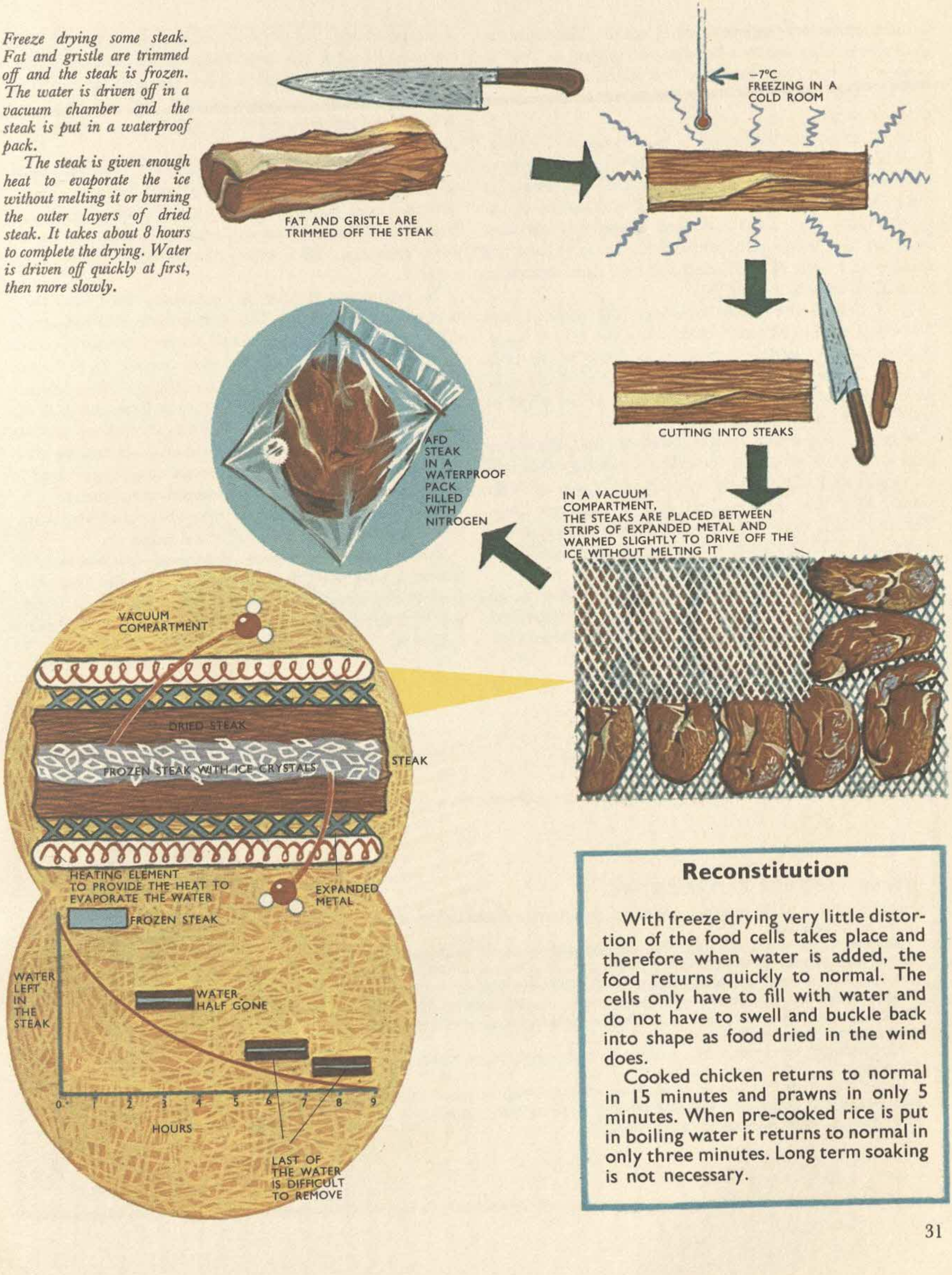
The main requirement is that no water must leak in. The food is therefore sealed in damp-proof packs. These must not be punctured during storage. Water vapour from the air will be taken up by the food, which once it contains water will go bad just as quickly as ordinary food.

Many foods will oxidize if there is any oxygen present. Fat tends to go yellow and rancid because of this and many vegetables develop a hay-like odour. Where experiments have shown that the food will not keep if there is oxygen present, the waterproof pack is filled with nitrogen instead.

The washing on the line has dried even though it was frozen solid. The water has evaporated off without the ice melting. It has frozen and dried – freeze dried.

Freeze drying some steak. Fat and gristle are trimmed off and the steak is frozen. The water is driven off in a vacuum chamber and the steak is put in a waterproof pack.

The steak is given enough heat to evaporate the ice without melting it or burning the outer layers of dried steak. It takes about 8 hours to complete the drying. Water is driven off quickly at first, then more slowly.


FAT AND GRISTLE ARE TRIMMED OFF THE STEAK
−7°C FREEZING IN A COLD ROOM
CUTTING INTO STEAKS
IN A VACUUM COMPARTMENT, THE STEAKS ARE PLACED BETWEEN STRIPS OF EXPANDED METAL AND WARMED SLIGHTLY TO DRIVE OFF THE ICE WITHOUT MELTING IT
AFD STEAK IN A WATERPROOF PACK FILLED WITH NITROGEN
VACUUM COMPARTMENT
DRIED STEAK
FROZEN STEAK WITH ICE CRYSTALS
STEAK
HEATING ELEMENT TO PROVIDE THE HEAT TO EVAPORATE THE WATER
EXPANDED METAL
FROZEN STEAK
WATER LEFT IN THE STEAK
WATER HALF GONE
0
1
2
3
4
5
6
7
8
9
HOURS
LAST OF THE WATER IS DIFFICULT TO REMOVE

Reconstitution

With freeze drying very little distortion of the food cells takes place and therefore when water is added, the food returns quickly to normal. The cells only have to fill with water and do not have to swell and buckle back into shape as food dried in the wind does.

Cooked chicken returns to normal in 15 minutes and prawns in only 5 minutes. When pre-cooked rice is put in boiling water it returns to normal in only three minutes. Long term soaking is not necessary.

remain active only where there is water. Although the scientific reason was not known, the drying of food to preserve it has been practised for thousands of years. Fish are split and hung up in the wind to dry; so are strips of meat.

Meat dried in the wind is nutritious and edible. Drying is effective in preventing decay, but the heat involved has a kind of cooking effect on the meat. As the cells lose their water they shrink and buckle out of shape. Once they have lost their shape it is not easy for them to return again, and if the meat is to have the texture and taste of fresh meat the cells must return to normal when water is added.

It is best if the water is driven off without any distortion of the cell walls, then when the food is being regenerated by adding water no swelling of the cells occurs. The water simply fills up each cell of the food which returns to its original state. This is the effect of accelerated freeze drying.

Whether in a melon or in a chunk of meat, the water content is situated inside the cells. A cabbage leaf will stay rigid and stiff because its cells are blown up with water. Dry the cabbage leaf and it becomes limp. The cell walls cave in as the liquid water evaporates away. But if all the water in the cells is frozen solid the cell walls do not collapse.

For this reason the food is first frozen before being dehydrated. For it to be effective the freezing must take place very quickly – it must be accelerated. When food is frozen slowly, large ice crystals form inside it. The ice crystals push the cell walls out of shape as they grow, and can completely spear through the walls so that liquid from a neighbouring cell can flow in. When the food is quickly frozen, the ice crystals are small and cannot do this.

The ice that has formed must then be removed without melting. Ice can evaporate and form a vapour without first forming a liquid. Washing that has frozen as stiff as a board will gradually dry out without ever thawing. The wind carries away the water vapour.

To encourage the ice to evaporate, the frozen food is placed in a vacuum. The water molecules evaporate off. Evaporation requires heat energy. As the first few molecules evaporate off they take energy of *sublimation* with them – energy required to change them from a solid to a vapour. This heat energy is obtained from the food which therefore grows colder. If nothing is done about it, evaporation will slow down considerably with the fall in temperature. Various techniques are used to apply heat to accelerate the evaporation of the ice.

After drying, the food is promptly sealed in dampproof packages to keep until it is needed.

Food which has been preserved by accelerated freeze drying should keep indefinitely. But, however perfect the idea, the process is never perfect. It is best not to leave the food for more than three years before eating it.

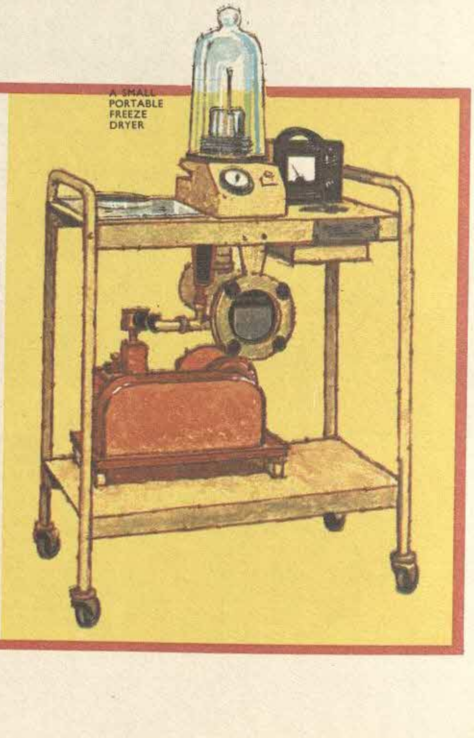
OTHER USES FOR FREEZE DRYING

Freeze drying is by no means confined to food storage although this is certainly its largest use.

Smaller freeze driers are used in hospitals and research establishments. At one time, human bone and skin for grafting could not be stored. After even small periods of time they deteriorated enough to become useless. Accelerated freeze drying has now made storage possible. Human plasma for blood transfusions, arteries, nerve plexus, and eye corneas for transplanting can also be preserved by freeze drying.

Pharmaceutical firms use this process for preserving many vaccines and antibiotics.

In the laboratory, specimens for viewing under the microscope or electron microscope can be freeze dried. The process prevents them from going bad and preserves them without distorting the cells.



A SMALL PORTABLE FREEZE DRYER

4. Bacteria and the soil

Bacteria and the soil

The growth of plants is dealt with elsewhere in this series. At this point three facts will be recalled: the first, that plants make complicated carbon compounds from carbon dioxide; the second, that plants absorb mineral salts called nitrates and use these in the manufacture of protein; the third, that the living part of plants consists of cells containing protoplasm which is largely protein. The diagram opposite illustrates the so-called nitrogen cycle. The repeated growth of green plants on a given area of soil would soon deplete the soil of nitrates if no mechanism existed for returning nitrates to the soil. The diagram describes some mechanisms. Here we are interested in the part played by bacteria. There is a chemical chain of events from proteins→ammonium compounds→nitrites→nitrates which take place in soil. Each link in this chain depends on one or other of the many soil bacteria.

Demonstration of ammonia formation from protein by soil bacteria

This work is in two parts: (I) Growing soil bacteria, (II) Using soil bacteria to break down protein.

Phase I

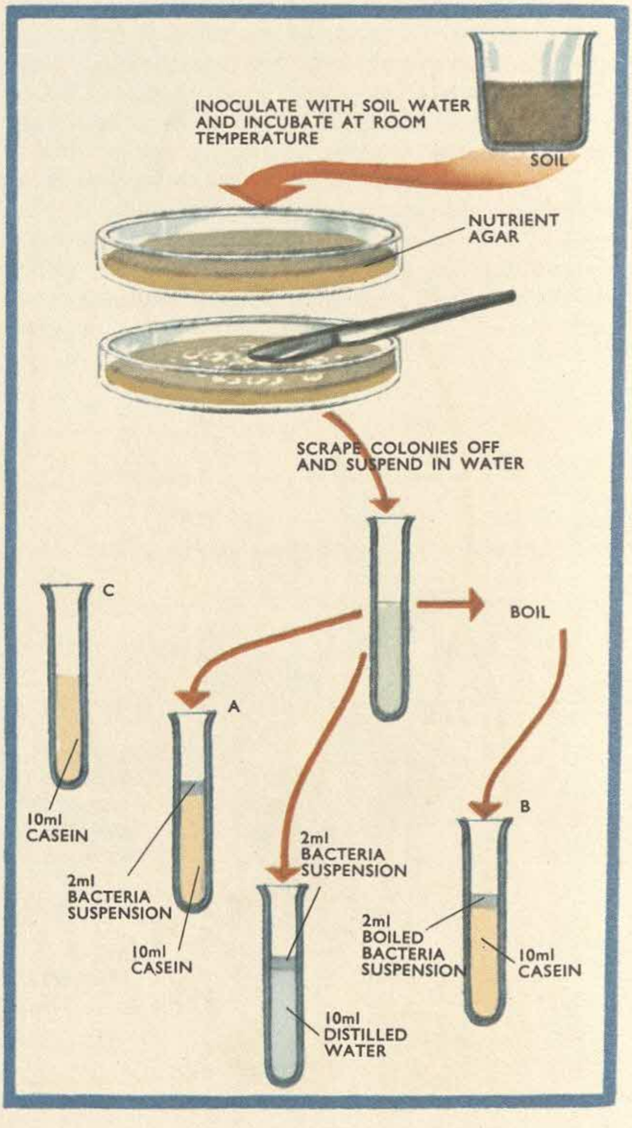
(1) Set up sterile nutrient agar plates in petrie dishes as described earlier. (See page 21.)
(2) Mix a heaped teaspoonful of rich garden loam with 50 ml. distilled water in a beaker.
(3) By means of a sterile instrument, e.g. a nichrome-wire loop, streak soil-contaminated water over the surface of the agar.
(4) Incubate at *room temperature*.

Phase II

(1) Make up in sterile glass a 2% solution of milk protein casein. (Use *recently* distilled water.)
(2) Put 10 ml. of 2% casein solution into each of three sterile tubes, A, B, and C.

Return to culture of soil bacteria after about a week's growth. By means of a sterile scalpel very carefully scrape a few colonies of bacteria from the surface of the agar. Suspend these bacteria in 10 ml. sterile distilled water in a sterile tube. Shake until suspension evenly distributed. Now add 2 ml. of suspension (sterile pipette) to lot A of casein, and 2 ml. of suspension to 10 ml. distilled water. Boil the remaining 6 ml. of suspension for at least 10 minutes – topping up with distilled water if necessary. Add 2 ml of boiled suspension to lot B of casein. Plug all tubes with sterile cotton-wool and incubate at room temperature for at least one week.

Now test each tube for ammonia by adding one drop of Nessler's solution. The latter, an alkaline solution


INOCULATE WITH SOIL WATER AND INCUBATE AT ROOM TEMPERATURE
SOIL
NUTRIENT AGAR
SCRAPE COLONIES OFF AND SUSPEND IN WATER
C
BOIL
A
B
10ml CASEIN
2ml BACTERIA SUSPENSION
2ml BACTERIA SUSPENSION
10ml CASEIN
2ml BOILED BACTERIA SUSPENSION
10ml CASEIN
10ml DISTILLED WATER

of potassium mercuric-iodide, gives an orange-brown colour with even dilute traces of ammonia. The depth of colour given is a measure of the amount of ammonia present.

This experiment shows that there are bacteria in soil capable of breaking down protein with the release of ammonia. The result of the Nessler's test will inform us which tubes contain ammonia. Any ammonia present in the bacteria and protein tube might have been in the casein at the beginning, or it might have been introduced with the bacteria. In order to prove that chemical changes which result in the formation of ammonia from protein are due to the activity of live bacteria, and, furthermore, to prove that these were soil bacteria, it was necessary to set up the experiment in the way described.

Explain carefully the information provided by the result in each tube.

The part played by other bacteria in the nitrogen cycle

The breakdown of proteins with the release of ammonia is but one of a number of chemical changes which have been shown to be due to the activity of soil bacteria. The conversion of ammonia to nitrites and nitrites to nitrates are two other examples of bacterial action known to occur in soil. Among the best-known bacteria involved in the nitrogen cycle are those found in wart-like nodules on the roots of plants in the pea family. Most soil bacteria live

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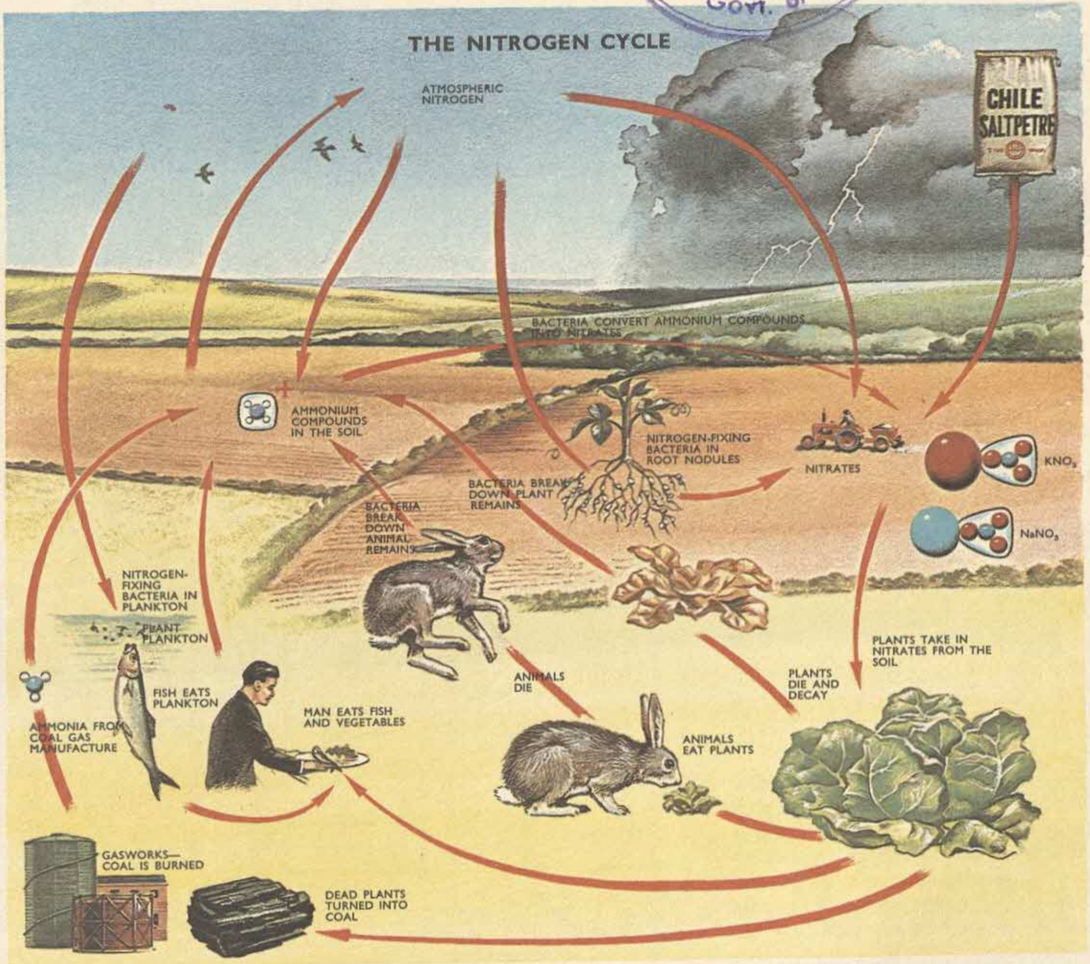

THE NITROGEN CYCLE
ATMOSPHERIC NITROGEN
CHILE SALTPETRE
BACTERIA CONVERT AMMONIUM COMPOUNDS INTO NITRATES
AMMONIUM COMPOUNDS IN THE SOIL
NITROGEN-FIXING BACTERIA IN ROOT NODULES
NITRATES
KNO₃
NaNO₃
BACTERIA BREAK DOWN PLANT REMAINS
BACTERIA BREAK DOWN ANIMAL REMAINS
NITROGEN-FIXING BACTERIA IN PLANKTON
PLANT PLANKTON
FISH EATS PLANKTON
AMMONIA FROM COAL GAS MANUFACTURE
MAN EATS FISH AND VEGETABLES
ANIMALS DIE
PLANTS TAKE IN NITRATES FROM THE SOIL
PLANTS DIE AND DECAY
ANIMALS EAT PLANTS
GASWORKS—COAL IS BURNED
DEAD PLANTS TURNED INTO COAL

free on soil particles, feeding on the organic matter in the soil. These 'root' bacteria have a special connection with the plants on which they live. Apparently the association, which is called symbiosis, is mutually advantageous to both plant and bacteria. The bacteria obtain carbon compounds from the plant; the plant obtains nitrogen-containing compounds from the bacteria.

The source of nitrogen used by these bacteria is nitrogen gas from the atmosphere.

Other bacteria – called de-nitrifying bacteria – break down nitrates and release nitrogen gas.

The fertility of soil depends on the activity of bacteria and on a balance of bacterial activity in favour of those processes leading to the formation of nitrates, over those leading to nitrate breakdown.

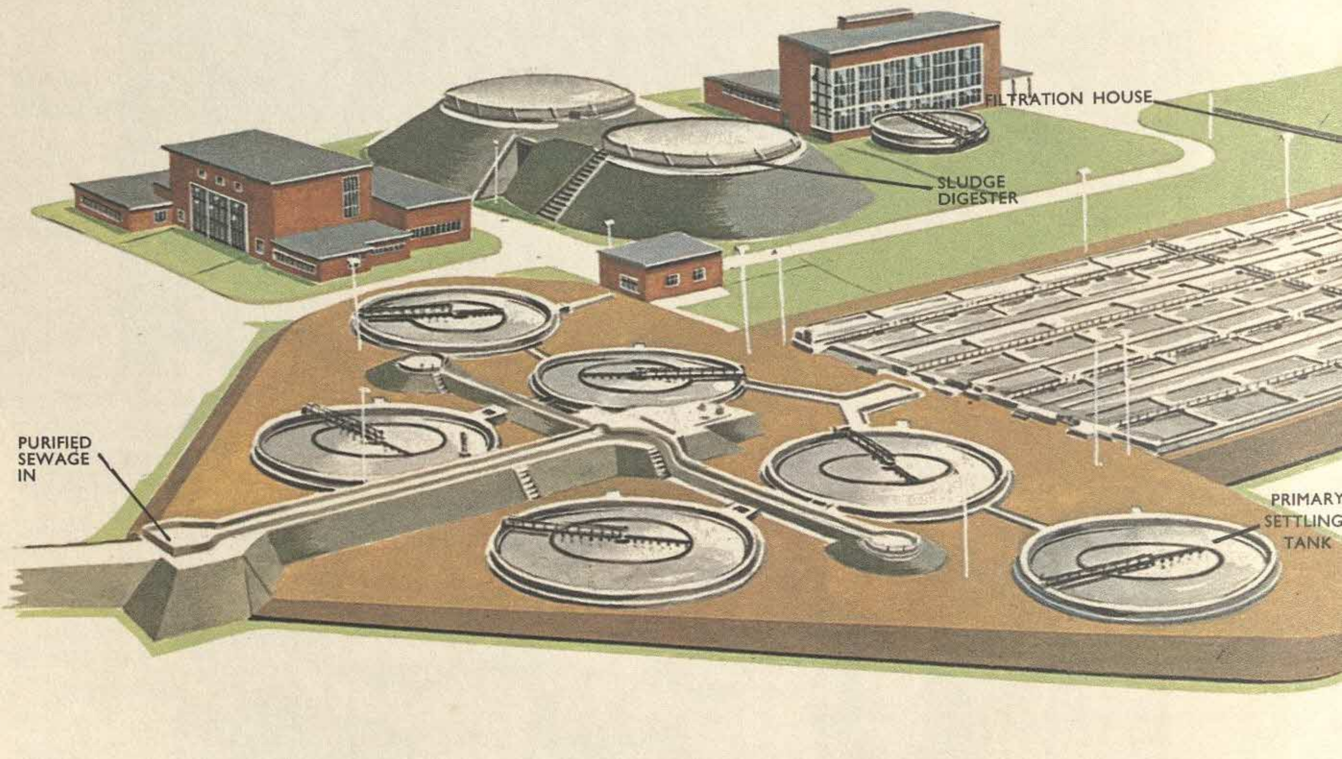
The maintenance of soil fertility is obviously important at any time, but especially in a world in danger of becoming over-populated. With this in mind, there follows a description of how the disposable waste of towns may be put to good use.

The purification of sewage

Sewage is the waste matter from toilets, kitchen sinks and baths, and the liquid wastes from industry. It may also contain surface water from gutters and street drains, depending on whether such fluids are piped directly into a river or to the sewage works along with other waste materials. All the drains connect with larger drains or *sewers* in the street, and these in turn link with *trunk sewers* to convey the liquid by gravity or by pumping to the *sewage works*. The network of sewers is known as the sewerage system.

Sewerage system

Water carriage systems were not introduced to this country until about 1870. Prior to that, household sewage was emptied into the streets or drained into open ditches at the sides of the street. Water supplies were frequently contaminated, and diseases spread rapidly, particularly those caused by water-borne organisms. Factories, also, discharged polluted water



A drawing of the Oxford Sewage Purification Works. The sewage dealt with here has already been screened and had the grit removed at a nearby unit. On the left can be seen the primary settling tanks. These feed into the aeration tanks (centre) and the final settling tanks (right). The large tanks beyond the primary settling tanks are the sludge digesters.

into streams and rivers, making the water in them unfit for use. Fish and other water life was killed by the pollution.

The need for purifying sewage and rendering it safe is obvious. But whereas sewage was once regarded merely as an obnoxious waste material which, once purified, was piped straight into the nearest river, today it is looked upon as a valuable raw water supply which must be conserved. Methane gas is recovered during the treatment of the slurry of solid material (sludge) that has been settled out from the rest of the sewage. The quantity of gas obtained in some sewage works is enough to produce all the power that is required. Methane has also been compressed and used to run vehicles, and it is possible that the sludge gas will be of value to the chemical industry, since such substances as methyl alcohol, formalin, acetylene and hydrogen can be made from methane.

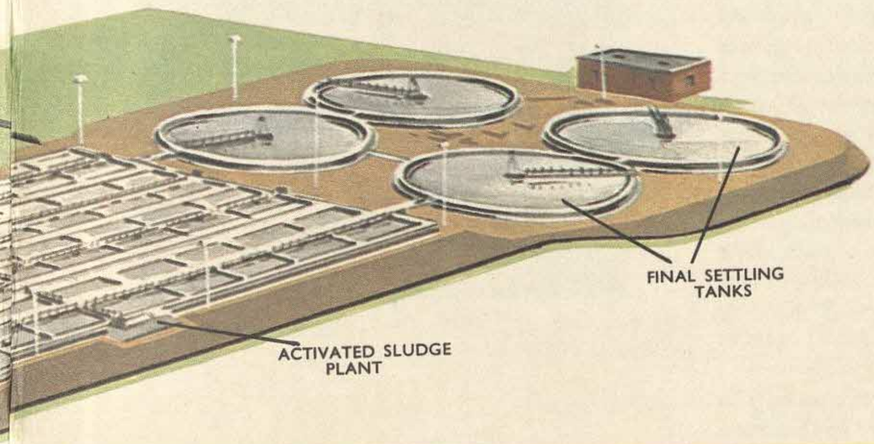
Sludge can be used as an agricultural manure or soil conditioner; it is a rich source of nitrogen and helps to develop the humus content of the soil.

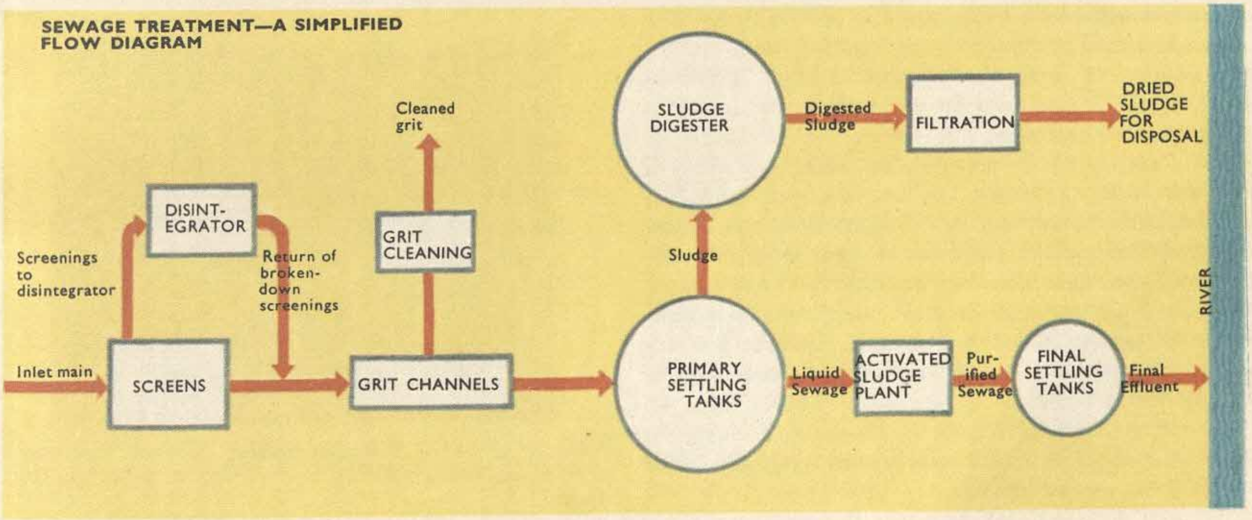
Sewage treatment

Sewage is rich in nutrients and in bacteria and other microscopic organisms (e.g. protozoans). If their growth is encouraged they can bring about remarkable chemical and physical changes. Indeed the process of sewage purification depends largely on the actions of micro-organisms, and improvements in the process have been concerned to a great extent with providing better conditions for their growth and increasing the efficiency of their actions.

Briefly, the main purpose of the purification is to allow harmless aerobic bacteria to oxidize the putrescible molecules into smaller harmless ones. Carbohydrates are broken down into carbon dioxide and water, whilst complicated nitrogen-containing molecules, such as proteins, are converted to ammonium compounds and then, by other bacteria, to nitrates.

Basically, sewage treatment consists of the following stages: (1) Screening and grit removal. (2) Settling or sedimentation. (3) Oxidation by micro-organisms (4) Sludge treatment.





Screening

Before the sewage passes to the settling tanks, where suspended solids settle out, it is usual to intercept rags, sticks, etc. with screens and also to pass the sewage through small tanks or channels in which grit is removed. In these the flow of sewage is maintained at about 1 foot per second, since this rate of flow is sufficient to keep the liquid sewage moving and not too fast to prevent the grit from settling out. To keep a constant flow at all times is a considerable problem since the rate of flow and type of sewage may vary. The grit is usually removed from the channels by dredgers, grabs or pumps. When washed it is suitable for use in land reclamation, building and repairing embankments and so on.

All sewage works have screens or some alternative device which sometimes come before and sometimes after the grit-removal step. The bars of the screens are usually ½ inch to ¾ inch apart so that solids of larger diameter than this are trapped. Upstream of these main screens there may be a set of coarser screens, the bars of which are about 4 inches apart. These intercept larger things such as bricks or timber. The waste matter collected by the screens is usually raked off them mechanically. It may be buried or burnt, though at some works it is cut up into fine pieces and returned to the flow of the sewage. Screening is not always used. Frequently a device called a comminutor is employed. The sewage is passed through this, the solids being cut up or ground down to small pieces which pass through narrow slots in the drum-shaped part of the comminutor with the rest of the sewage, thus avoiding the separate collection and dumping of such solids.

Settling or sedimentation

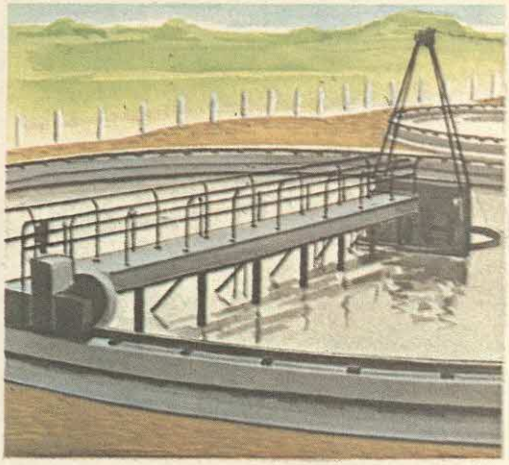
After removal of grit and screening, the sewage is piped or channelled to the settling tanks. In these the suspended impurities settle out and are removed as a liquid sludge. The sewage in the settling tanks moves very slowly – so slowly that suspended particles sink to the floor of the tanks. As far as possible, the sludge is removed mechanically, by revolving scrapers in circular tanks, or in rectangular tanks by scrapers that move up and down the length of the tanks. In circular tanks the sewage is fed in at the centre of the tank and flows upwards and outwards to overflow weirs all round the edge. The sludge collects in or is scraped to the centre of the tank floor. In horizontal flow tanks (e.g. rectangular tanks) it is scraped towards hoppers in the floor, usually at the inlet. Where possible, the settling tanks are kept in operation all the time or closed down for only short intervals and sludge is removed mechanically. Manual labour is expensive and slower than mechanical labour.

In those works that receive industrial sewage of a special nature the finer suspended solids (colloidal particles) are settled more quickly by adding chemicals such as aluminium sulphate.

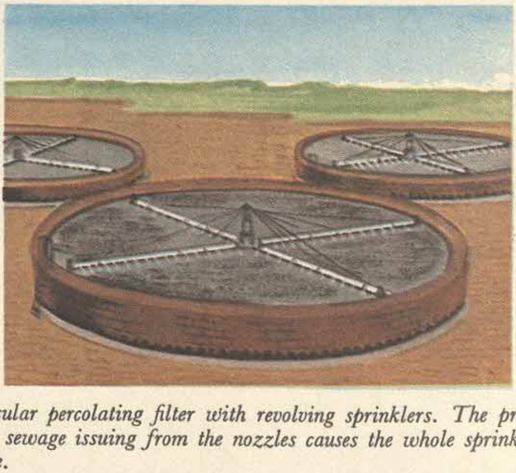
The bulk of the particles of solid matter is removed after sedimentation, but tank effluent requires further purification. This is carried out by bacteria and the like in special filters or in activated sludge plants.

Oxidation by micro-organisms

Whatever the method used, the liquid is brought into close contact with atmospheric oxygen and the



A circular primary settling or sedimentation tank with a revolving mechanical scraper.



A circular percolating filter with revolving sprinklers. The pressure of the sewage issuing from the nozzles causes the whole sprinkler to revolve.

right types of bacteria and other micro-organisms. At one time land treatment of sewage was commonly used. This method is rarely employed in urban areas, but is still common in many parts of the world.

The sewage is left in the open air to be decomposed by the actions of microbes. Such sewage farms require large areas of flat land, however, and often these do not dry off rapidly enough to permit ploughing and replacement of air in the soil surface.

Percolating filters are more commonly used for small towns, whereas activated sludge units have so far proved more economical for larger towns, though quite small installations are becoming available. The beds of percolating filters are made of pieces of coke, clinker or stone. The liquid sewage is sprayed over these pieces or 'media' and forms a film of jelly over each of them. The bacteria and other organisms grow in this jelly and break down the chemical impurities in it. The spray of liquid flows continually through the filter beds, washing away excessive amounts of the jelly, and thus ensures that air has free access into the bed. The effective surface area of the porous mass of media in each bed is enormous and so each filter bed is able to handle large quantities of sewage. Because the organisms require free oxygen to carry out their chemical actions they are said to be aerobic. In some works purified sewage is fed into the filters with unpurified sewage, increasing the flow of liquid through the filter and also reducing the time it takes to purify a given volume of sewage. Two sets of filters may also be used, the partly purified sewage being fed into a second set of filters after leaving the first.

Activated sludge tanks in which the liquid is agitated by paddle wheels. This agitation introduces air into the liquid, encouraging the growth of micro-organisms.

Filter units may be circular or rectangular and the sprays may be fixed, may revolve – the pressure of the liquid turning them just as water does in a revolving garden sprinkler – or they may move backwards and forwards along rails where filters are rectangular.

The purified liquid issuing from the filter is normally clear, though it contains some solid – mainly small particles broken off the pieces of media in the filter beds. These solids are separated in a further set of settling tanks or humus tanks and the fluid (final effluent) is discharged into a nearby river.

In the activated sludge system air is blown up through the sewage as small bubbles or introduced at the surface by agitating it with stirring devices. The bacteria are added to the sewage in the activated sludge, a brown muddy liquid that is prepared by aerating the sewage itself. Several hours' aeration of sewage, to which activated sludge has been added, is sufficient to purify it. When the sludge settles out later it is pumped back to the aeration tanks to purify more sewage. Excess sludge is removed from time to time and passed to the sludge processing plant.

These operations require power; and though an activated sludge plant takes up less room than percolating filters and can be made more attractive in lay-out, it is more expensive to run. The sludge produced is also very liquid, so increasing the difficulties of treating it. More efficient aerating devices have been developed, however, which enable larger volumes of sewage to be purified in tanks of the same size.

On the long term, an activated sludge plant is more economical.

Sludge is separated in settling tanks similar to those described earlier. This part of the activated sludge treatment of sewage is equivalent to the humus tank stage of the filter treatment.

Sludge treatment

Sludge from the settling and separating tanks is a thick, muddy liquid. Many methods are used to process and dispose of it. Often it is digested in special digesters. These are large tanks in which the sludge is heated to 80–90°F. and acted upon by anaerobic bacteria (bacteria that do not require atmospheric oxygen). Much of the solid material is broken down and valuable methane gas is produced. The sludge is reduced in bulk, its fetid smell eliminated and the remaining solids hold less water. It is then easier to handle, drain or dry.

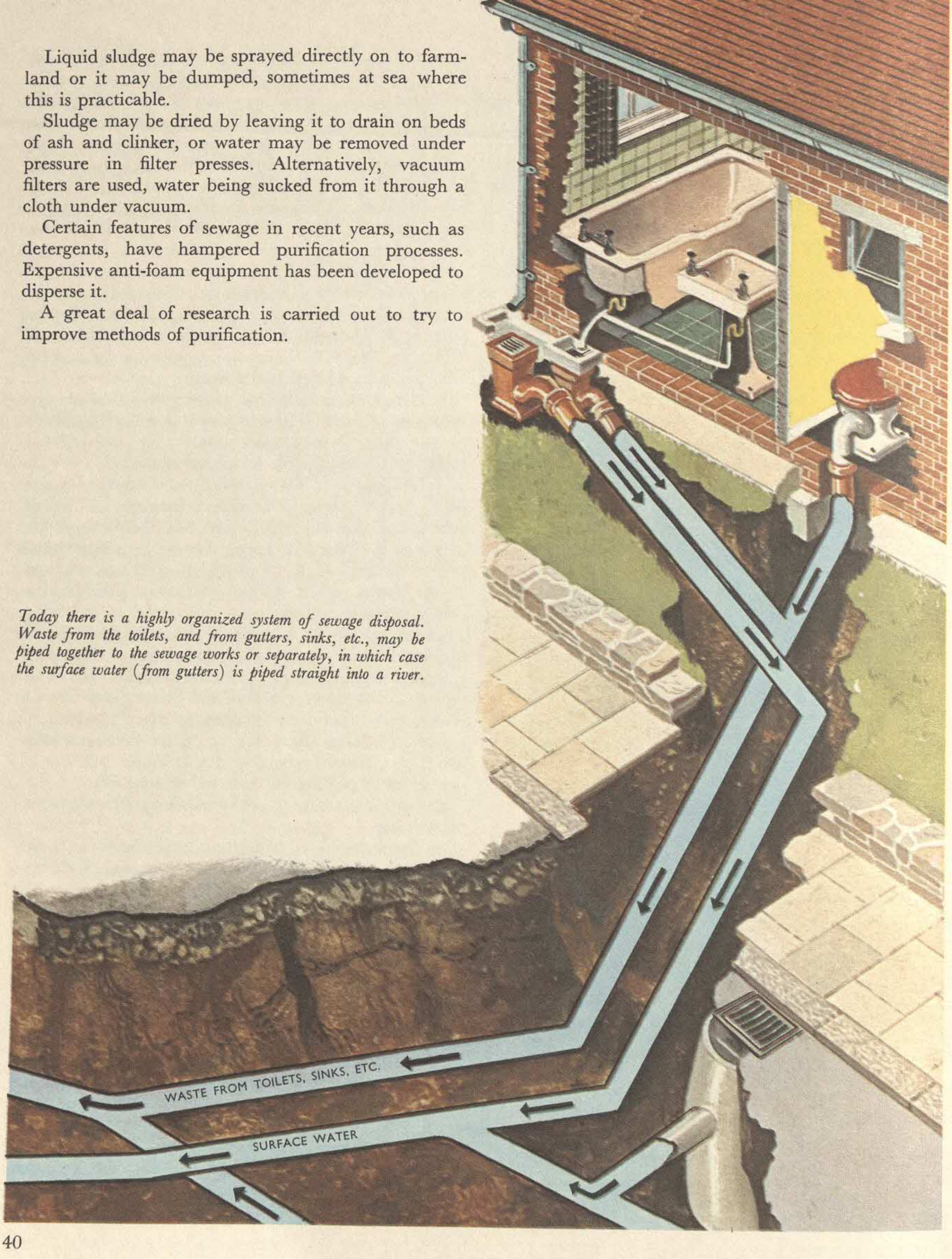
Liquid sludge may be sprayed directly on to farmland or it may be dumped, sometimes at sea where this is practicable.

Sludge may be dried by leaving it to drain on beds of ash and clinker, or water may be removed under pressure in filter presses. Alternatively, vacuum filters are used, water being sucked from it through a cloth under vacuum.

Certain features of sewage in recent years, such as detergents, have hampered purification processes. Expensive anti-foam equipment has been developed to disperse it.

A great deal of research is carried out to try to improve methods of purification.

Today there is a highly organized system of sewage disposal. Waste from the toilets, and from gutters, sinks, etc., may be piped together to the sewage works or separately, in which case the surface water (from gutters) is piped straight into a river.


WASTE FROM TOILETS, SINKS, ETC.
SURFACE WATER

5. Disease-causing organisms

Disease-causing organisms

It was not until the nineteenth century that the great French scientist Louis Pasteur showed that many diseases were caused by minute organisms called bacteria. He also discovered ways of adding to the body's natural resistance to some diseases.

Before Pasteur's work no protective measures were known against many diseases. Many great plagues swept across the Earth. During the Great Plague of 1665 in London it is estimated that over one hundred thousand people died out of the population of under half a million. In the Black Death of the fourteenth century about twenty-five million Europeans died – a quarter of the population.

What is disease?

Any disorder or unhealthy condition of the body is called a disease. Many of the diseases which affect us are caused by bacteria, viruses and protozoans (single-celled animals). Some others are caused by fungi and others by worms (e.g. tapeworms and hookworms). In addition there are, of course, many physiological disorders.

Bacteria

Well over a thousand different kinds of bacteria are known. Relatively few cause disease. The smallest bacteria are but 1/25,000 inch in diameter.

Diseases Due to Bacteria

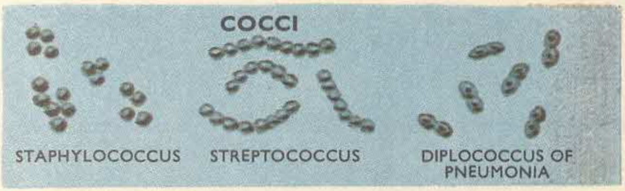
Disease	*Type of Bacteria*
Diphtheria	Bacillus
Typhoid	Bacillus
Tuberculosis	Bacillus
Bubonic plague	Bacillus
Tetanus	Bacillus
Leprosy	Bacillus
Whooping Cough	Bacillus
Scarlet Fever	Streptococcus
Meningitis	Streptococcus and Bacillus
Pneumonia	Diplococcus, Bacillus, Streptococcus and Staphylococcus
Erysipelas	Streptococcus
Blood poisoning	Streptococcus
Boils	Staphylococcus
Relapsing fever	Spirillum

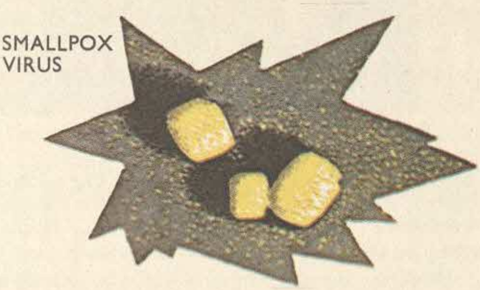
Disease-producing bacteria are called pathogens. Pathogens are responsible for many diseases of man. The injurious effect of pathogenic bacteria on the body tissue is due to the chemical substances they release during their growth or on their breakdown after they die. These substances are called toxins and are generally characteristic of a given type of bacterium producing a characteristic disease. The tubercle bacillus, for example, gives rise to tuberculosis and never to diphtheria or anthrax; the typhoid bacillus produces typhoid or enteric fever. However, some diseases, such as pneumonia and meningitis, may be produced by a number of different organisms.

Pathogens may enter the body through wounds in the skin, by being breathed in (after being sneezed or coughed out by some other infected person), in food, milk, or water, or via insects. Tetanus, for example, is brought about by the entrance of the tetanus bacterium in dirt through wounds; diphtheria, scarlet fever and measles may be breathed in; dysentery and enteric fever are caught by eating the unclean foodstuffs containing the bacteria causing these diseases; whilst insects, such as fleas, carry and pass on the plague bacillus.

Viruses

Viruses are responsible for many diseases, such as poliomyelitis, smallpox, influenza, yellow fever, chickenpox, mumps and measles. The largest are only 1/80,000 inch in diameter and the smallest 1/2,500,000 inch in diameter. They appear to be on the borderline between living and non-living things. The simplest appear to be very like crystals; yet they can absorb other substances, grow and reproduce by splitting in a way which is not fully understood at present. When a virus multiplies within a cell it may cause the death of the cell. It appears that in some cases the virus inserts itself in the natural workings of the cell, using them to manufacture copies of itself. Some viruses seem to produce ill effects in a certain type of tissue only. The viruses which cause poliomyelitis and





rabies, for example, attack nerve cells. Those that cause influenza and the common cold affect cells in the breathing system.

Viruses can only grow and multiply in the living cells of an animal (or plant). They depend for their survival upon being passed on from one living thing to another. They may be carried in the air from one person to another during normal breathing out, coughing and sneezing. The yellow-fever virus is carried by a certain kind of mosquito and passed on to humans when they are bitten by the mosquito. Viruses nearly always gain entry through the skin (particularly through cuts) or through the wet lining of the mouth and throat.

Protozoans

Several single-celled animals (protozoans) cause diseases in humans. One, a relative of *Amoeba* called *Entamoeba*, causes dysentery, an infection of the bowel wall. Another, a trypanosome, causes sleeping-sickness. This protozoan is carried by the tsetse-fly and is passed on to a human host when the fly pierces the human skin to suck up the blood on which it feeds. The same protozoan is also able to live in game animals, but it appears to have no ill effects on them. This clearly shows that disease-causing organisms often produce the disease in one kind of animal only.

Perhaps the best known of the disease-causing protozoans is the malaria parasite (*Plasmodium*) which causes malaria. This organism spends part of its life in a certain mosquito. A human is infected when he is bitten by this mosquito.

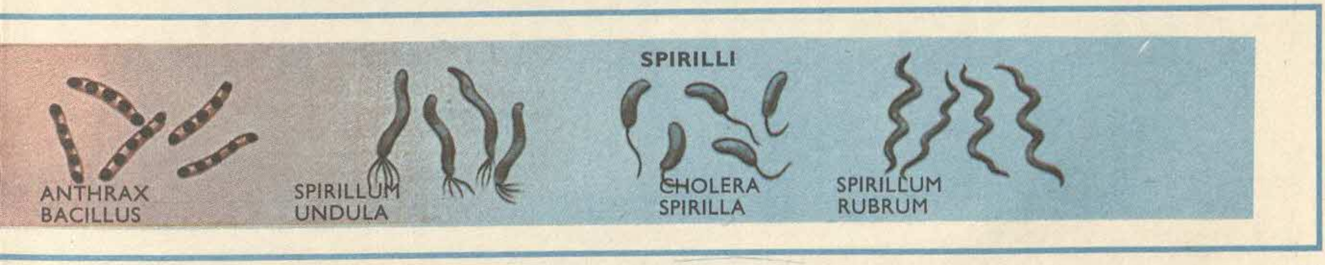
TWO KINDS OF INFLUENZA VIRUS

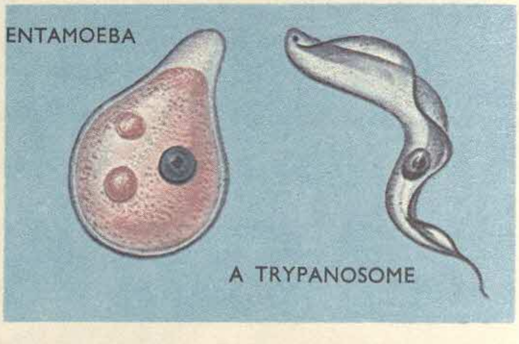
Some Diseases Due to Viruses

Influenza
Measles
Mumps
Smallpox
Yellow Fever
Poliomyelitis (polio)
Hydrophobia (rabies)
Chickenpox

Worms

Many kinds of worms cause diseases in man. These fall into three main groups: flukes, tapeworms and roundworms. Some have extremely complicated life-cycles affecting two or three different animals (each called a host). The Chinese liver fluke, for example, lives in snails and fishes at different periods in its life-cycle. It affects man when he eats raw fish, as is a common practice in parts of China and Japan, and finds its way to the liver where it feeds on blood. Another fluke, a blood fluke or schistosoma, has only one host, apart from man, a fresh-water snail. The adult female fluke lays her eggs in the blood vessels of the human intestine. The eggs pass to the outside world in the faeces. If they reach water they hatch as small larvae which swim about in the water until a snail is encountered. Inside the snail they feed on the body tissues and each is changed into a sac (called a sporocyst). From each sporocyst





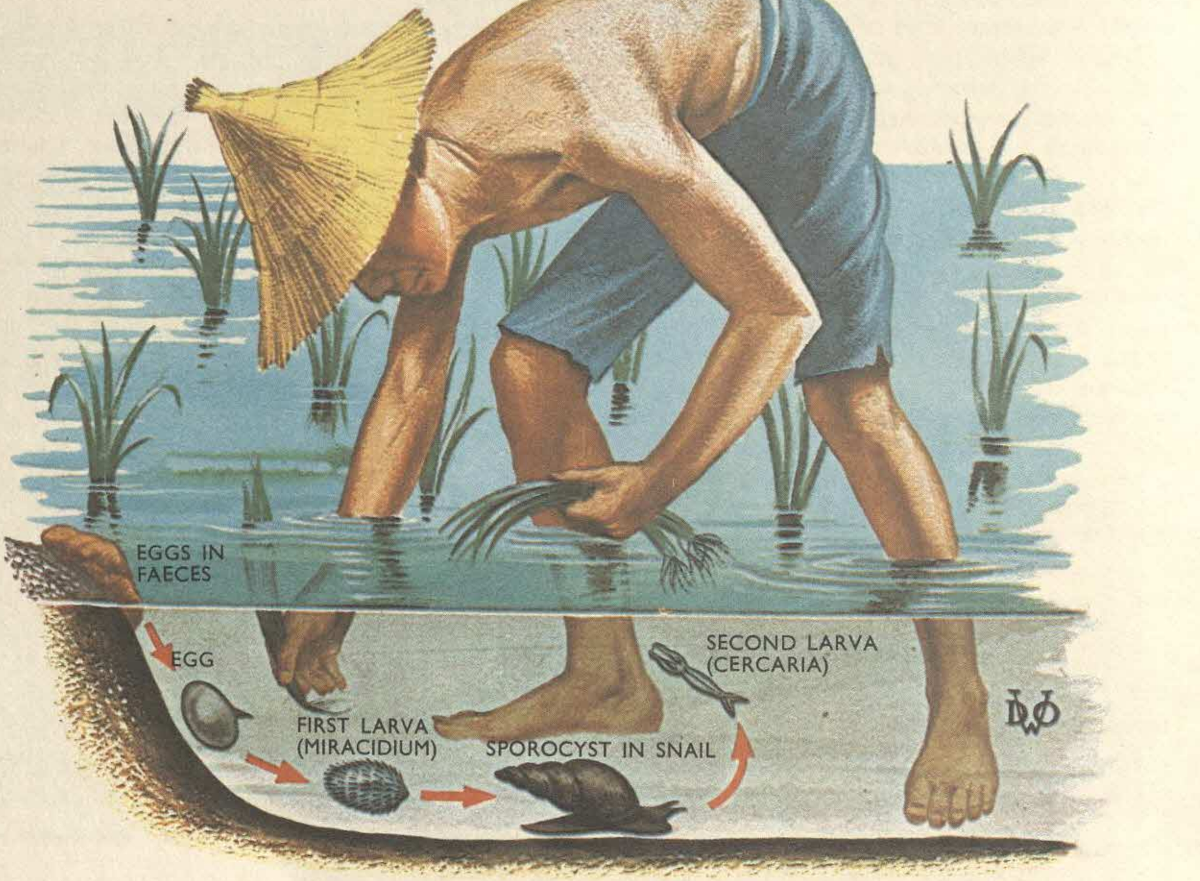
Entamoeba, *a relative of* Amoeba, *lives in the human intestine feeding on the lining cells, and often causes dysentery. One kind of trypanosome which causes sleeping-sickness is carried by the tsetse-fly which passes it on to a human host when the fly bites the skin.*

many larvae of another type emerge. They leave the snail and swarm in the water. If they come into contact with the bare skin of a person who is washing or bathing in water infected by them they bore through the skin until they reach a blood vessel. They are then carried to the intestine where they grow into adult flukes. The disease produced (called schistosomiasis) is very common in China and in Egypt. It weakens the body considerably after a time and so may itself cause death or leave the body very susceptible to other diseases. In Japan and China the disease is easily obtained when the rice is being planted, for the planters stand bare-legged in the waterlogged paddy-fields which are fertilized with human faeces.

Several adult tapeworms infect man. One has the pig as its other host, another infects cattle, and a third infects fish. A human may become infected when he eats raw or undercooked pork, beef or fish.

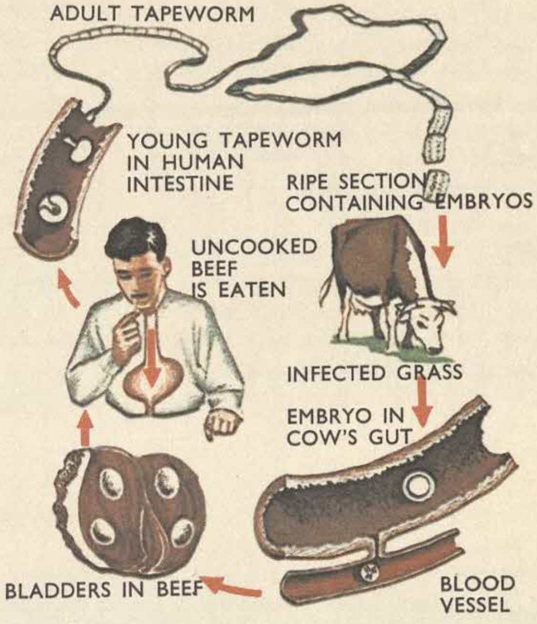
Fortunately, only a few of the roundworms which infect man cause serious diseases. One is a tiny hookworm about three-eighths of an inch long called *Necator*. The adult lives in the intestine, hanging on to the lining with its mouth and sucking blood. The eggs laid are passed out in the faeces and, where sanitary conditions are poor, fall onto the soil. They hatch into larvae which grow and feed in the soil

The life history of a blood fluke.



until they reach a certain size. If they come into contact with bare skin they are able to burrow through the flesh, passing through the blood vessels or other channels to the lungs. From here they find their way up the windpipe into the mouth and are swallowed into the stomach, then passing into the intestine. Hookworm disease is common in many tropical and subtropical countries. Individuals become lazy and lifeless and children are often mentally backward.

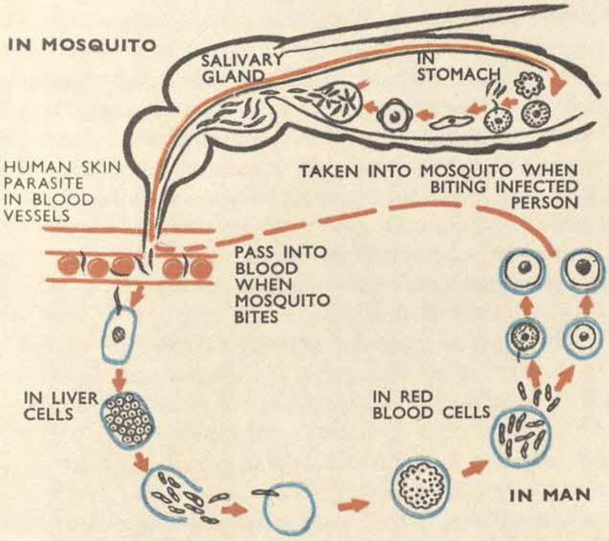
Trichina worm, one of the most harmful worms, is less than an eighth of an inch long. The adults live in the intestine for a while and the female bores through the intestine wall into tiny channels called lymph vessels. The young worms are born here and are carried about the body in the lymph and the blood. Eventually they invade the muscles, where they increase rapidly in size before they form a protective wall round themselves. Unless the flesh of the host is eaten the worms eventually die. Humans usually become infected by eating undercooked pork. The larvae cause great damage and pain by burrowing into the muscles and many die from the disease which is called trichinosis.

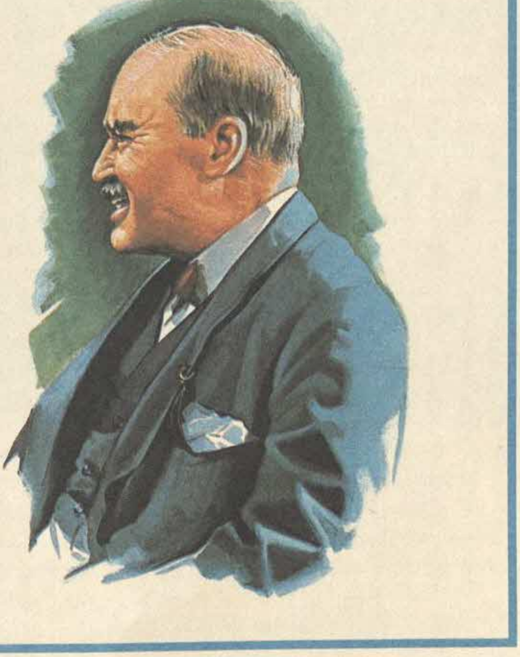


The life history of a tapeworm.

Sir Ronald Ross

The connection between the disease malaria – the organism which causes it – and the insect which transmits it is an interesting example of scientific detection. The credit for finding this must go chiefly to Sir Ronald Ross (1857–1932), a British doctor working in India. Encouraged by Sir Patrick Manson, an eminent authority on tropical medicine, Ross spent a great deal of time and energy trying to track down a definite link between malaria and the mosquito. After years of intensive work examining all kinds of mosquitoes under a microscope, he discovered in one of them, a female *Anopheles,* an unusual kind of cell. On further investigation the cell was found to contain a few granules of black pigment, exactly like that of the malaria parasite in man. This was the proof of the link which Ross had been seeking so long. The mosquito had been identified and so had the means by which it transmitted the disease to man.


IN MOSQUITO
SALIVARY GLAND
IN STOMACH
HUMAN SKIN
PARASITE IN BLOOD VESSELS
TAKEN INTO MOSQUITO WHEN BITING INFECTED PERSON
PASS INTO BLOOD WHEN MOSQUITO BITES
IN LIVER CELLS
IN RED BLOOD CELLS
IN MAN



One other roundworm of tremendous importance is the *Filaria* worm. The adult filarias live in the lymph channels, blocking these and causing great swelling of the affected part – a condition called elephantiasis. The worm is spread by mosquitoes which suck up the young larvae from the blood vessels and pass them on when they bite another person.

Fungi

Diseases caused by fungi are not very common in man. Ringworm is a disease of the skin which is so called because it is often characterised by ring-shaped wounds. It may affect the hair.

The life cycle of the malaria parasite

The infected mosquito injects many parasites into the blood stream. Most of them probably die but some reach the liver where each enters a cell. For ten days approximately (according to species), the parasite grows and multiplies in the liver. As yet the infected person feels no ill-effect. Each parasite divides into perhaps several thousand tiny individuals which are released when the liver cell ruptures.

Some of these new individuals enter other liver cells and repeat the cycle, others enter red blood corpuscles where they again multiply. The parasites can be seen and identified under the microscope at this stage. Each parasite divides within the corpuscle and produces twenty or so new individuals. The corpuscle then breaks up and releases them. They enter new corpuscles and the process starts again. Every 48 or 72 hours the parasites are released from the corpuscles. The destruction of corpuscles results in anaemia and the release of the millions of parasites produces the bouts of fever.

In some corpuscles the parasites produce special forms which perish unless taken into the stomach of a feeding mosquito. Here, 'male' and 'female' parasites join up and form a cyst in the stomach wall. Hundreds of new mobile individuals form in the cyst. When they are released they move through the body and some enter the salivary glands. It is from here that they re-infect man when he is bitten by the insect. The cycle in the mosquito takes about twelve days

Mosquitoes and malaria

Two hundred million people – that was the number of people suffering from malaria some years ago. This disease must surely be the greatest scourge of mankind, but it is one that is being conquered. Twenty years ago malaria was common throughout most of the tropics. It has now been eradicated from many countries, and the World Health Organization is aiming to eradicate malaria for good. Even so, it is still estimated that it causes about one million deaths annually in India alone.

The word 'malaria' means 'bad air' and reflects the idea that it was associated with the mists of marshland. This connection is only indirect, as it has been known for a long time that the disease is spread by mosquitoes. These insects, of course, breed in water and are always common on marshland. The earlier name for malaria was *ague*.

The disease

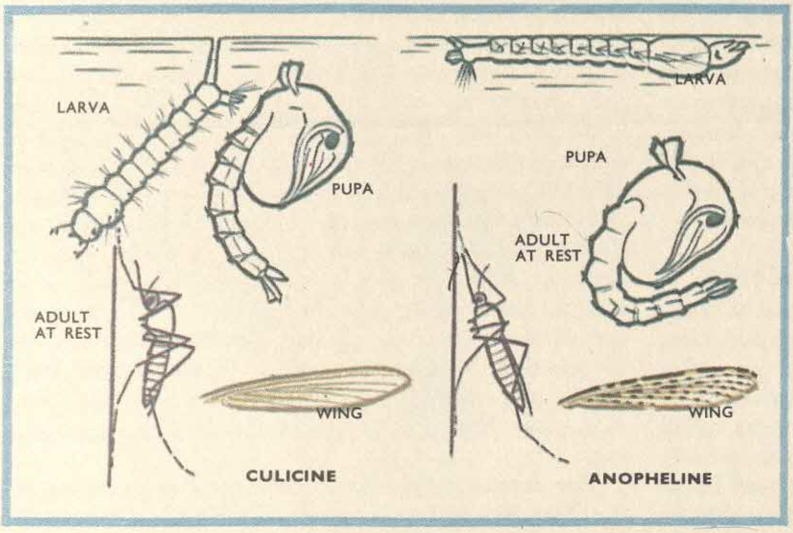
Malaria is caused by infection with minute protozoans of the genus *Plasmodium*. These tiny parasites live and multiply in the red blood corpuscles. Periodically the corpuscles burst and release the parasites. This produces the periodic fever and anaemia characteristic of malaria.

The disease is carried from man to man only by certain kinds of mosquito (*Anophelines*). The mosquito picks up the disease organisms when it takes blood from an infected person, but it cannot pass them straight on to another. The parasite has to undergo a series of changes inside the mosquito before it can be passed on to another human. These changes take some days.

Anopheline mosquitoes occur all over the world, but malaria is only prevalent in those regions where the temperature is about 70°F. or more. The malarial parasite does not develop in the mosquito at much lower temperatures. Many species can carry the malaria parasite, but some are more important *vectors* than others. The term 'vector' is applied to an organism (usually an insect) that transmits disease from one animal to another. The term 'carrier' refers to an animal (e.g. man) carrying the disease germs but not displaying symptoms.

The malaria parasite takes some days to complete its changes in the mosquito. If the mosquito dies before this period is up, it will not be able to transmit malaria. Mosquitoes that bite man only rarely will also not be important vectors. Only a small proportion of mosquitoes will actually live long enough to pass on the disease, and so malaria is only prevalent where there are large populations of mosquitoes.

The commonest form of the disease is *malignant*



The life cycle of the mosquito

Only the female mosquito feeds on blood. Without it she cannot produce fertile eggs. After pairing she takes a blood meal every few days. The eggs are laid in little 'rafts' on the surface of some suitable water. The tiny larvae hatch in a day or so and live in the water, feeding on microscopic materials. They breathe air through tiny tubes that pierce the water surface. When fully grown (after a week or two) the larvae turn into pupae. The pupae are unusual in that they swim actively, although they do not feed. The adults emerge from the pupae in a day or two.

There are two main groups of mosquitoes – *Culicines* and *Anophelines*. Only the latter can carry malaria although many other diseases are transmitted by Culicines and Anophelines alike. The adults differ in wing pattern (no spots in Culicines) and in their resting positions. The larvae also differ in their resting positions under the surface film.

tertian malaria. It is also the most serious form. The parasite is *Plasmodium falciparum* and it is restricted to warm and tropical countries. After someone has been bitten by an infected mosquito it takes about two weeks (varying with temperature) to produce symptoms. These are bouts of fever – hot and cold sensations with sweating. The fever corresponds with the increases and spread of the parasites in the blood. The bouts of fever do not return regularly in this type of malaria and they may be almost continuous. Complications are common, notably clotting of the affected blood corpuscles. This is especially serious when clots occur in the blood-vessels of the brain. A patient who survives the initial infection usually has occasional relapses, with irregular bouts of fever, until the disease disappears.

Plasmodium vivax causes *benign tertian malaria*, which is less serious than the malignant form and slightly less common, although it occurs in cooler as well as tropical regions. After an incubation period of about two weeks the fever occurs and returns every 48 hours for five or six weeks. It then becomes irregular. Relapses may be caused by cold or exertion. Eventually the disease disappears altogether. The parasites may be in the body, however, for up to three years. People carrying the disease (carriers) in this way are difficult to detect.

Quartan malaria is similar to benign tertian malaria except that the fever occurs every 72 hours.

Treatment and control of malaria

Quinine is the traditional drug for treating malaria, but it is not highly effective. It suppresses the symptoms but does not kill the parasite, and relapses occur unless the drug is continued. *Paludrine* and other drugs have superseded quinine. A small daily dose kills off any parasites that may be acquired. Larger doses are used to cure an infection already in action.

The control of malaria can be achieved in two ways: by attacking the parasite or by attacking the mosquito vectors. Malaria can also be avoided by choosing living quarters away from mosquito breeding grounds. The treatment of patients with drugs is sound in theory, but in practice it does not give good control of the disease as a whole. So many people are carriers of the parasite without knowing it that there is always a reservoir of infection. Only if all carriers were treated could the disease be eradicated with drugs alone, and this is economically impossible.

The most efficient way of protecting a community is by attacking the mosquito population – either as larvae or as adults. To attack the breeding sites and prevent breeding and growth of larvae is often very costly and is justified only in areas of high human population or where the mosquito breeding sites are well defined. Control of breeding involves drainage of wet areas, biological control of the larvae with insect-eating fishes, or the use of insecticide.

The first large-scale use of insecticides against adult mosquitoes was in about 1935. Pyrethrum insecticide gave very good results. DDT, Lindane (γ–BHC) and other synthetic insecticides are now extensively used as *residual insecticides*. They are sprayed in and around houses and their effect is long-lasting. When insects rest on a treated surface they pick up a dose of insecticide and die within a given period. Not all the mosquitoes will be killed at one time, but it is unlikely that any will survive the two weeks necessary for the changes to be completed in the malaria parasite. This is the important point. No mosquito will be able to *transmit* malaria.

Eradication schemes are using this fact now. If spraying goes on for a few years, there will be no transmission of disease and during this period all or most of the patients will undergo natural cure or cure by drugs. There will then be no more malaria parasites in the area and the disease will have been eradicated. Even when spraying is stopped the disease will not reappear. It is not usually necessary to control breeding of the mosquitoes as well to achieve this result.

Sleeping-sickness and the tsetse-fly

African sleeping-sickness is one of the most dreaded of tropical diseases and occurs in a broad belt stretching across the middle of the continent. It is one of a number of diseases – affecting man and other animals – that are caused by minute protozoan parasites called *trypanosomes*. Infection with these parasites is technically called *trypanosomiasis*. A lot of the poverty and backwardness in Africa has been ascribed to the existence of this disease, for it prevents man and his animals from occupying what would otherwise be good land.

There are many species of trypanosome, but not all of them are harmful to their hosts. Rats, sheep and many others are often infected but show no signs of ill health. Most kinds of trypanosomiasis occur only in tropical or sub-tropical areas. Examples include sleeping-sickness (Africa), Chagas' disease (tropical America), nagana (an African disease of animals) and surra, a serious disease of hoofed animals and others, occurring throughout the tropical and sub-tropical regions. Almost all trypanosomes are transmitted by insects.

African sleeping-sickness

This disease exists in two different forms – Gambian (caused by infection with *Trypanosoma gambiense*) and Rhodesian (caused by *T. rhodesiense*). The Gambian form is commoner in the north and west of the region and the Rhodesian form in the south and east, but they overlap to some extent. It is believed that man is the only host of *T. gambiense*. The Gambian form of sleeping-sickness is the less severe of the two, and affected people continue to work for a time; they are then an important source of re-infection. Rhodesian sleeping-sickness is a much more rapid and severe disease. Affected people usually remain in their homes, away from the tsetse-flies which transmit the disease. These people are not important sources of infection. The parasites exist (apparently without doing any harm) in various game animals, and man picks them up by the bite of a fly when out hunting.

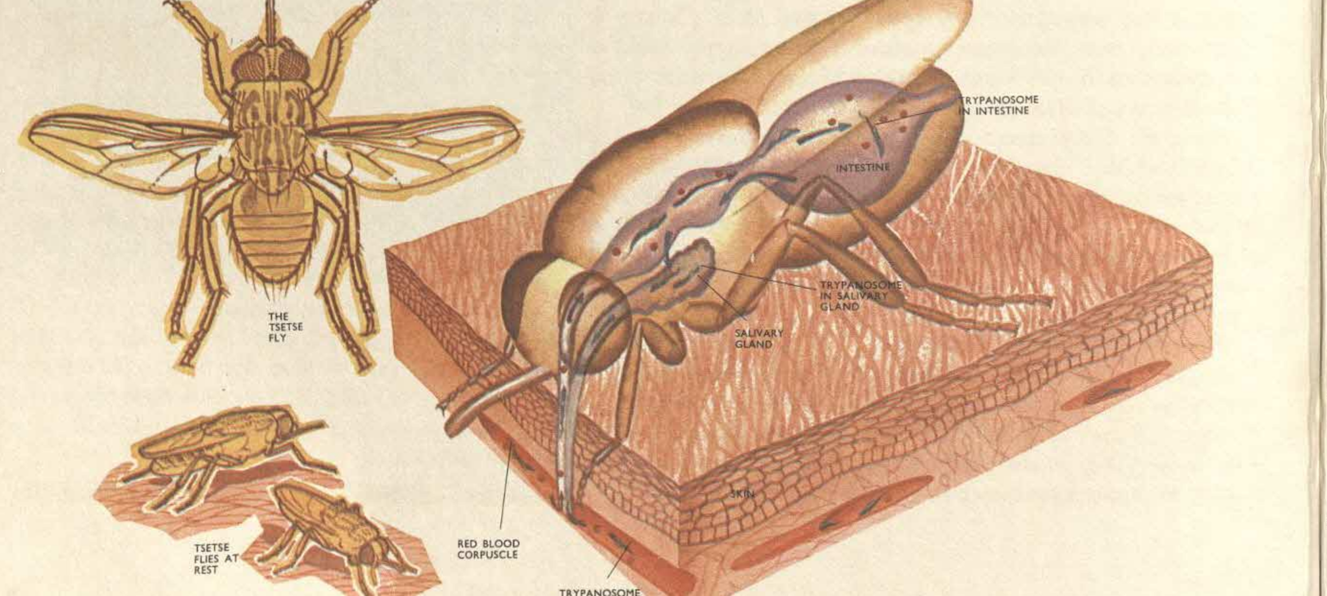
The disease is spread mainly by species of tsetse-fly. Parasites enter the mammal's blood-stream when the fly takes a feed of blood. The region of the bite becomes inflamed, and this is followed by fever and sickness as the parasites multiply in the blood-stream. Loss of weight is considerable, especially in the Rhodesian form of the disease. Later, the trypanosomes enter the brain and spinal cord and produce tremors and lethargy – hence the name 'sleeping-sickness'.

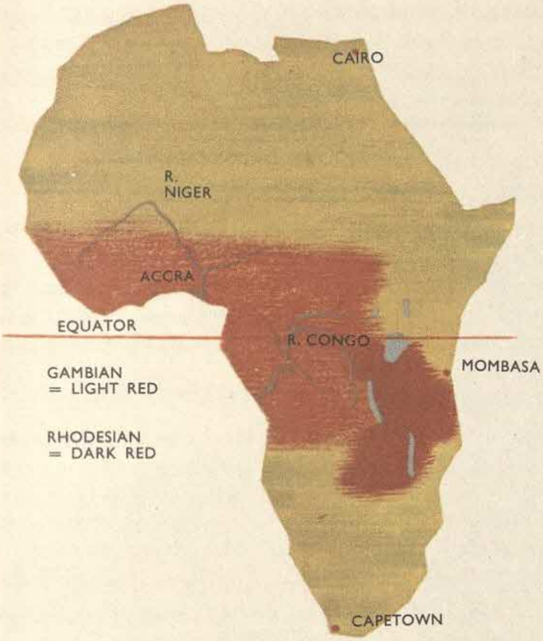
Unless treated early on, sleeping-sickness results in death. The various stages may last some months (even years in the Gambian form) before death occurs. Drugs of various types (including compounds of arsenic) have effected cures in some cases, and all infections are probably curable if treated early enough.

Transmission of sleeping-sickness

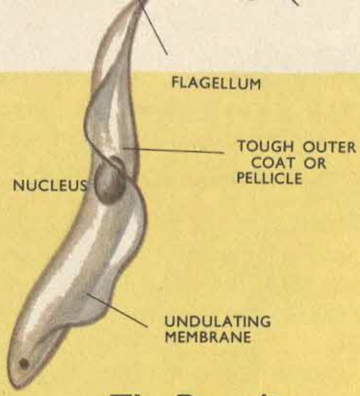
The disease is spread mainly by tsetse-flies of the genus *Glossina*. Both sexes of these insects feed on

The tsetse-fly shown diagrammatically feeding on an infected host. Parasites enter the host from the fly's salivary gland. Fresh parasites enter the fly from the host's blood-stream.





The distribution of Gambian and Rhodesian sleeping-sickness.



The Parasite

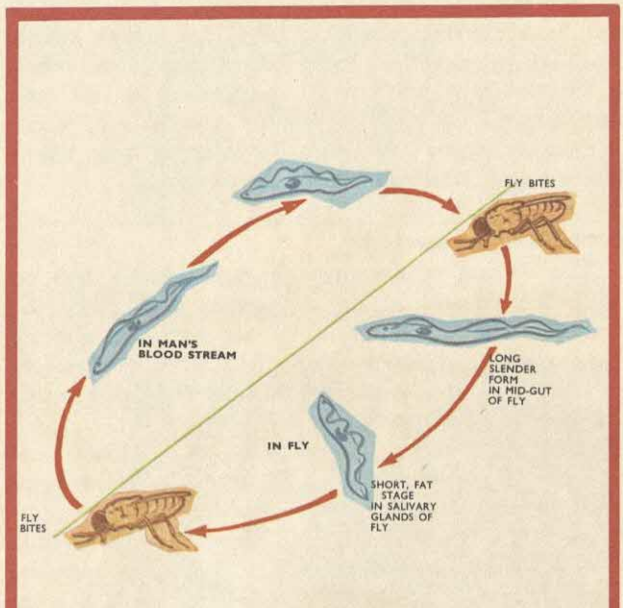
Trypanosomes are minute protozoans of the class *Flagellata*. The typical form is illustrated but variations occur during the life history. Trypanosomes move freely in the blood of the vertebrate host by means of the flagellum and undulating membrane. Food and oxygen are absorbed through the tough coat (pellicle) and waste products leave by the same route. Reproduction is by simple division into two or more individuals, thereby allowing a large population to build up quickly.

blood and they take up the parasite when they feed on the blood of an infected person or animal. If the meal is interrupted and the fly begins to feed on another person it is possible for parasites adhering to the blood-sucking organ to pass directly to the new host. Other blood-sucking insects can also transmit trypanosomiasis in this way. In general, however, trypanosomes cannot be transmitted directly. They must undergo changes within the body of a tsetse-fly before they can re-infect a vertebrate host.

Sleeping-sickness trypanosomes pass along the insect's food canal with the meal of blood. Many of the parasites are destroyed by the digestive processes, but some survive and establish themselves in the mid-gut. There they multiply rapidly, and within two or three weeks – according to temperature – trypanosomes appear in the salivary glands of the insect. The fly is then able to pass on the parasites when it next takes a meal. During its spell in the insect the trypanosome passes through several stages. In the mid-gut the parasite is usually long and narrow. Those reaching the salivary glands are shorter and fatter; these are the forms that re-infect man.

Not all trypanosomes follow the same cycle of events as the sleeping-sickness parasites. *T. brucei* – one of the nagana-causing parasites – resembles the

Life cycle of Trypanosoma. *Stages take place in the tsetse and in the human host.*


FLY BITES
IN MAN'S BLOOD STREAM
LONG SLENDER FORM IN MID-GUT OF FLY
IN FLY
SHORT, FAT STAGE IN SALIVARY GLANDS OF FLY
FLY BITES

sleeping-sickness organisms, but *T. vivax* – another nagana-causing parasite – does not develop in the mid-gut. To complete the cycle the parasites must become attached to the inside of the blood-sucking organ (*proboscis*). They multiply there and then get into the salivary duct (hypopharynx), through which they enter another vertebrate host. *T. congolense* also causes nagana, but it undergoes a period of development in the mid-gut before reaching the proboscis and salivary duct.

Chagas' disease is caused by *T. cruzi*, but it is transmitted by blood-sucking bugs, not tsetse-flies. The parasites develop in the hind-gut of the insect and pass out with its faeces. They enter the human host through cuts and sores on the skin. A similar life-history is found with some trypanosomes carried by tsetse-flies.

The tsetse-fly

Tsetse-flies belong to the genus *Glossina*. They are confined to Africa but occur over most of that continent south of the Sahara. They are, however, restricted to certain situations such as open woodland around stretches of water. They tend to avoid villages and open spaces. *Glossina palpalis* is the main vector (insect carrier) of Gambian sleeping-sickness, while the Rhodesian form is carried mainly by *G. mortisans*. This species also carries *T. brucei*, which causes nagana in cattle.

Tsetse-flies are a little larger than house-flies and are brown in colour. They are biologically peculiar in that they do not lay eggs. Every ten days or so the female brings forth a full-grown larva which has been nourished by secretions of the uterus. When it leaves the parent's body the grub does not feed but immediately starts to burrow into the soil, where it forms a pupa. The adult fly emerges some weeks later to a life of almost daily blood-sucking.

Controlling the tsetse-fly

The control of sleeping-sickness is closely tied up with the control of the tsetse-flies that carry the disease. Several species of fly are involved and all have slightly different habits. Control is therefore difficult and none of the methods tried so far has met with complete success.

Insecticides have reduced tsetse-fly numbers considerably in some areas, but this method is expensive and by itself not altogether satisfactory. When combined with bush clearance it has been successful. Clearing scrub around lakes and rivers has removed the shade so necessary for the tsetse-fly, and this has made many areas habitable by man. *Glossina mortisans* has been controlled in some regions by destroying antelopes and other game on which the insect depends largely for its food. Clearing of vegetation and human settlement seems to be the most satisfactory way of controlling the tsetse-fly.

Viruses

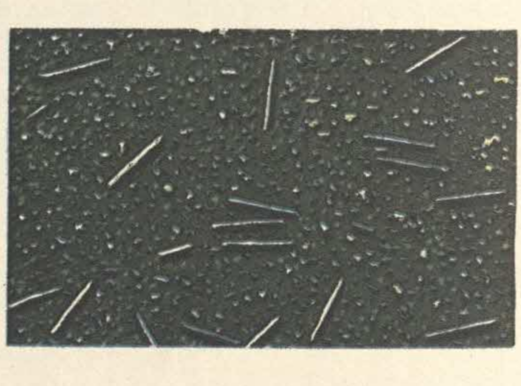
Viruses appear to be on the borderline between living and non-living matter. They are minute infective particles so small that the majority can be seen only with the electron microscope. Their sizes range between 10 and 300 millimicrons (1 millimicron $(m\mu) = \frac{1}{1,000,000}$ millimetre). In gross structure a virus might be compared to a potato in its jacket, the jacket being a shell of protein surrounding the potato – a core of nucleic acid; the latter is either ribonucleic acid (RNA) or desoxyribonucleic acid (DNA). The polio virus has this typical structure.

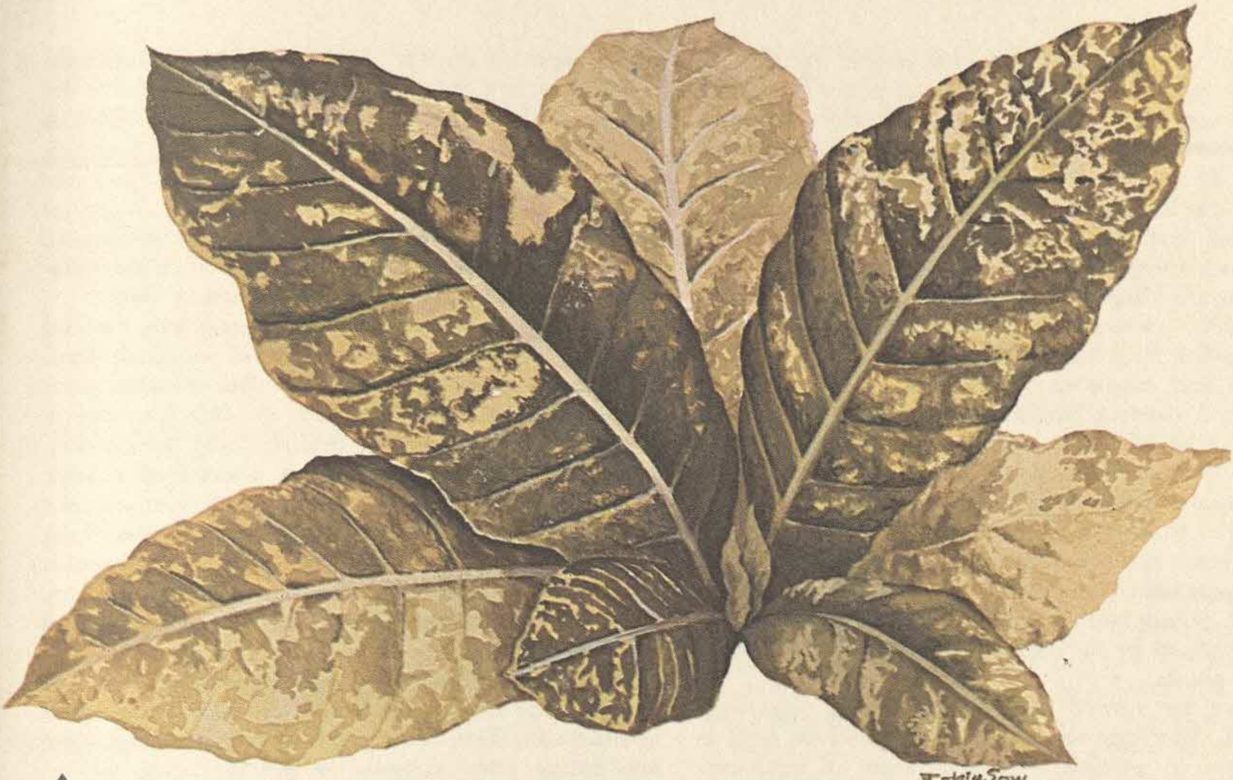
Nucleic acids are chemical compounds that occur in the nuclei and cytoplasm of both plant and animal cells. In the nuclei, combined with protein, they are arranged in strings on the chromosomes and form the basis of the hereditary material whose 'instructions' regulate all the activities of an organism.

Viruses are the smallest known 'packets' that contain all the information required for their own reproduction. They can only grow and reproduce at the expense of living cells. The shell is a protective outer coating that may be used to penetrate the host cell. The core enters the cell and uses the chemicals within it to produce more virus 'packets' complete with shells (the cell may be completely destroyed). Eventually the cell wall breaks and the viruses are released.

Though viruses are so small, it is by consuming the contents of the cell and disorganizing its normal workings that the symptoms of the many virus diseases

Tobacco mosaic viruses are rod-like structures about one four thousandth of a millimetre long (magnification here × 30,000).

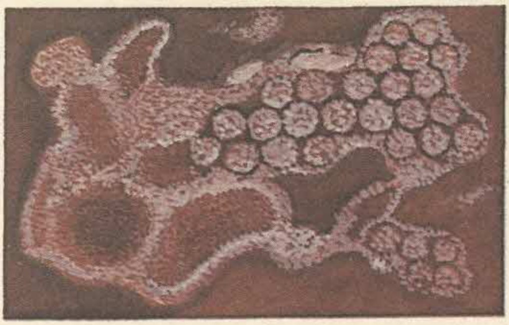




A tobacco plant infected with tobacco mosaic virus.

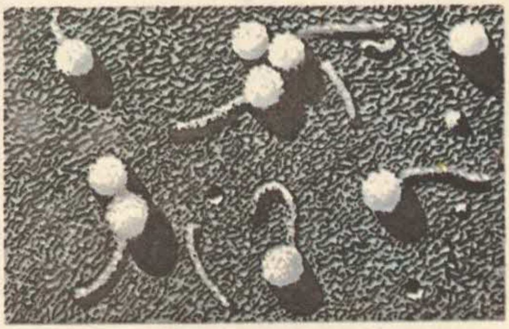
are produced. Poliomyelitis, smallpox, yellow fever, chicken-pox, measles, influenza and the common cold are virus diseases affecting man. Foot-and-mouth disease affects cattle, sheep, and pigs, and fowl-pest affects poultry. Myxomatosis, caused by myxoma, affects rabbits. It was introduced into Australia in 1951 in an attempt to control the plague of rabbits there, and later was introduced into Great Britain with the same object.

Poliomyelitis virus particles, magnified many thousand times, shown inside an infected cell.



Aucuba mosaic viruses are like long, thin fibres. They cause mosaic disease in tomato plants.

This bacterial virus only attacks bacteria. It has been photographed with the electron microscope and is magnified many thousands of times.



Many plant diseases are also caused by viruses; potato mosaic, tobacco mosaic, potato-leaf roll, cauliflower mosaic, turnip yellow mosaic and sugar-beet yellows are examples. Such diseases are characterized by certain symptoms. In potato and tobacco mosaic the leaves are mottled; with sugar-beet yellows the whole leaf often appears yellow.

Viruses are spread in similar ways to many bacteria. Their control depends to a large extent on the manner in which transmission occurs. Lettuce mosaic is transmitted from one plant to another in the seed. Insects and eelworms (a kind of roundworm) are important carriers, transmitting viruses from diseased plants to healthy ones. Aphids (e.g. green-fly), thrips, flea-beetles and leaf-hoppers are carriers of economically important viruses that attack plants. For example, the virus causing sugar-beet yellows is spread by a black bean aphid and the potato and peach aphid. The latter also transmits potato-leaf roll and potato mosaic. Potato mottle is not thought to be transmitted by insects but by the contact of healthy and unhealthy potato plants.

There are several ways of controlling sugar-beet yellows. The sugar-beet seedlings should be kept as far away as possible from root crops of sugar-beet and mangels. Sowing the seedlings late helps to make this isolation more effective, and insecticides sprayed over them kill aphids arriving on the new crop. Heavily infected crops may have to be destroyed completely.

Potato diseases are partly avoided by using fresh seed-potatoes obtained from special seed-potato-producing areas that are relatively free from infection by insect-carried viruses.

Insects are also prominent as carriers of animal viruses. Perhaps mosquitoes and fleas are the most important in this respect. Viruses may also be spread from one animal to another by direct contact, spitting or coughing. Colds or influenza may be spread in this way. Poliomyelitis viruses are transmitted by way of infected faeces, blowflies being probable carriers. Smallpox is very contagious because the virus escapes from breaks in the skin.

Insects that transmit diseases

Ever since man became a social animal, gathering together to live in communities, the medical problems of combating germ-caused disease have increased. Today our troubles are added to by the ever-increasing ease of communication between once isolated parts of the world. Wherever man lives with his fellows in large numbers the problems of providing clean food, adequate sewage disposal facilities, household refuse-collection services, and so on, are considerable. Many of these facets of our life provide habitats for disease-causing organisms (pathogens) and breeding grounds for insects, a number of which are responsible for spreading disease. The organisms that cause malaria, yellow fever, dysentery, sleeping-sickness, filariasis, bubonic plague, typhus, poliomyelitis, kala-azar, and yaws are among those carried by insects.

From all kinds of dirt, refuse, sewage, badly drained soil etc., insects are contaminated by pathogens. These may be picked up by their feet or other parts of the body as they crawl over the infective source, by the mouthparts during feeding or by swallowing. When the insects frequent places where food is kept, or when they come into contact with humans, the pathogens may then be passed on. Blood-sucking insects (e.g. mosquitoes) may take in pathogenic organisms while feeding upon an infected person. When they feed again, the pathogens may be passed on to someone else.

The many kinds of insect each have their own way of life – their food, habitat and life-cycles may differ considerably. Thus some are carnivores, preying upon and eating other animals; others are herbivores or scavengers, and many live as parasites, permanently on, or in close association with, hosts. The manner in which they feed also differs considerably. Thus some have piercing mouthparts that penetrate animals and plants in order to suck up their juices; some have biting mouthparts, and others merely suck up their food. The adults may only come out at night (e.g. cockroaches), hiding in dark, moist places during the daytime. Some adults are long-lived, others live for only a few days. The larvae of some live in water, others in the soil or in plants or animals, and others under decaying vegetation.

Obviously, then, if an insect is considered to be a carrier or *vector* of disease-causing organisms, all aspects of its life and anatomy must be known thoroughly. The parts of the body that carry the pathogens must be investigated; the life-cycle of the pathogen within the body (in appropriate cases) must be studied; any other animals that can act as temporary carriers must be discovered. Only when these and numerous other facts are known can adequate control measures be formulated. Possible breeding grounds can be eliminated, and contact between the insects and man can be reduced by using repellent creams and by placing nets over beds. The use of insecticides may also eliminate the insects or at least reduce them to a more tolerable level.

6. Preventing and curing disease

Preventing disease

Once man stopped regarding disease as the result of a 'visitation by the Devil' and started to see symptoms as the effects of a cause which could be tracked down, prevention and cure became possible.

The application of knowledge which we now possess about the cause of certain diseases makes possible their prevention. This knowledge also helps in the control of diseases – control of spread and control of effects.

Consider the case of a bacterium which causes a disease in man. From the bacterium's point of view its first problem is that of gaining entry into the body. Now the body is covered with a bacteria-proof layer of skin, so the number of places where entry is possible is small. These are:

1. Entry with inspired air via lining of mouth and respiratory tract – i.e. trachea, bronchi and lungs.
2. Entry with food via alimentary tract.
3. Entry via cuts or wounds which have damaged the skin surface.
4. A rather special case of (3) through punctures caused by other organisms, especially insects.

The techniques used for preventing infection relate to these pathways into the body. Obviously if it were possible to remove the bacterium from all the air breathed and from all the food eaten, to prevent damaging the skin surface, to avoid contact with organisms able to pierce skin, then an individual so protected would not get the disease. The measures taken to minimize the risk of bacterial infection may be classified on the basis of the above ideas, as follows: firstly, the treatment of food, including methods of preservation, cooking, pasteurisation; secondly, the treatment of water supplies; thirdly, sanitation techniques which ensure that organic waste is separated from food and air supplies; fourthly, disinfecting – i.e. treating possible sources of bacterial infection with chemicals known to kill bacteria; fifthly, eradication of possible insect carriers of bacteria; sixthly, eradication of secondary hosts of bacteria.

Two properties of bacteria make possible control of infection. These are: (a) their susceptibility to high temperature, (b) their sensitivity to certain substances.

Temperature and bacterial infection

Prolonged boiling, especially under pressure (i.e. at temperatures greater than 100°C.), kills the majority of bacteria. Some bacteria are killed at lower temperatures when they are applied for some time – e.g. 62°C. applied for 20 minutes kills the bacteria which cause tuberculosis. Low temperatures do not usually kill bacteria but slow down their life-processes so that they cannot grow or reproduce.

Chemical sensitivity

Several substances are known to be lethal to bacteria at concentrations which do not harm man. One such substance is hypochlorous acid. These substances are called disinfectants.

For such uses as the sterilisation of milk bottles, where the only aim is to kill all germs, strong and severe methods can be used. Gentler methods are needed for cleaning wounds, however, and disinfectants for such uses are called antiseptics. Joseph Lister was the first to treat wounds in this way.

JOSEPH LISTER

Surgery with safety

Although the introduction of anaesthetics in the mid-nineteenth century made possible a large number of operations, the survival rate of patients was very low. The reason was not bad surgery, but bacterial infection of the tissues from the instruments and the surroundings. Such infection caused decomposition of the tissues, blood poisoning and death. Nowadays, every part of the body can be operated upon in complete safety. The development of the *antiseptic method* (the forerunner of modern *aseptic surgery*) was Lister's contribution to medical science.

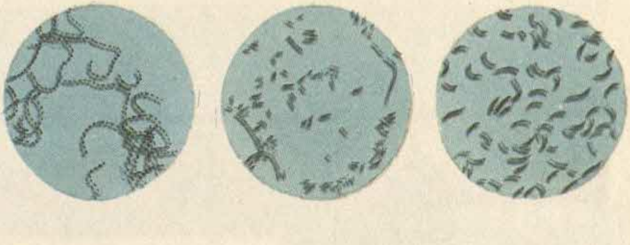
The hospital wards of which he was in charge had a bad record of gangrene and other similar infections. Lister continued his study of wound healing and inflammation and noted that simple fractures (those where the bone does not break through the skin) healed relatively easily. Compound fractures, where the broken bone is exposed, were often fatal. Many people had thought that the formation of pus, and the putrefaction of the wounded tissue, was caused by the air itself, but Lister showed that this was not the case. He suspected that something carried in air was the cause.

A colleague at Glasgow drew Lister's attention to the work of Pasteur in France. Pasteur had shown that the souring of milk and wine was due to the action of tiny living organisms (bacteria) that were always present in the air. Lister deduced that the putrefaction of wounds was also due to micro-organisms and he set about finding a means of destroying them. Throughout his work Lister always acknowledged his debt to Pasteur and the two men became great friends.

Treatment of water supplies

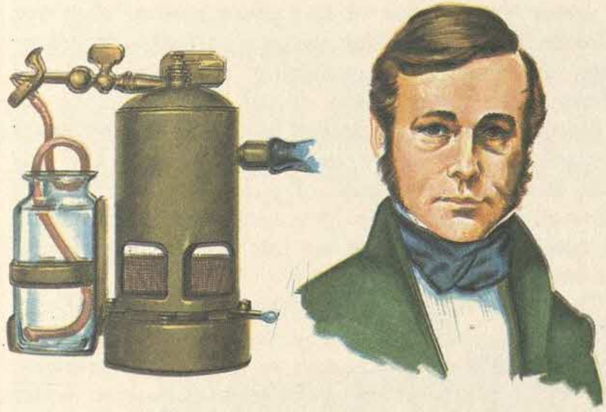
Water supply is an essential feature of everyday life and is more or less taken for granted in civilized communities. Water companies are required by law to provide pure water, free from visible suspended matter, free from taste and smell and from any mineral or organic matter which could be harmful to health or detrimental to industrial processes. When one considers that in a typical town more than fifty gallons are provided each day per head of population, one can appreciate that this is no small undertaking.

Water is obtained usually from rivers, but, in areas where the geological formation allows it, wells (sunk into water-bearing rocks) may provide a substantial contribution to the volume of water required. (Wells in the London area provide 15% of the Metropolitan Board's water, the rest comes from the Thames and its tributaries.) River water is usually muddy and contains much undesirable matter, including millions of harmful bacteria, all of which have to be removed before the water is considered pure enough to go into the service mains leading to your kitchen tap. Many towns have no suitable river from which they can obtain water. In such cases water will have to be piped – often for great distances – before reaching the town's waterworks. Sometimes a whole valley may be dammed to produce a semi-natural lake which will act as a storage reservoir from which water can be taken. This has been done in Westmorland to provide a water supply for Manchester, many miles away.



Some bacteria found in river-water.

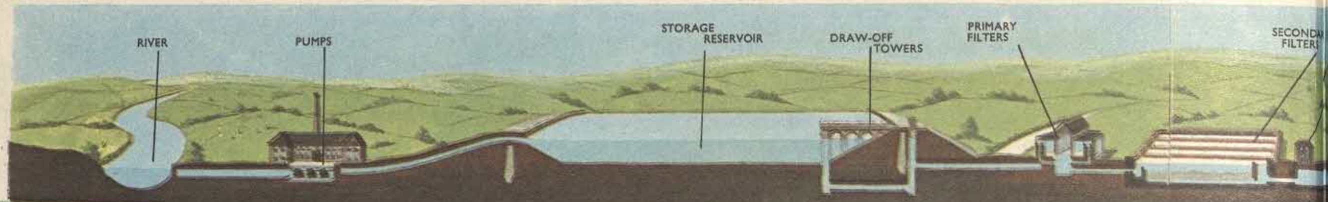
STATE INSTITUTE OF EDUCATION
Govt. of West Bengal

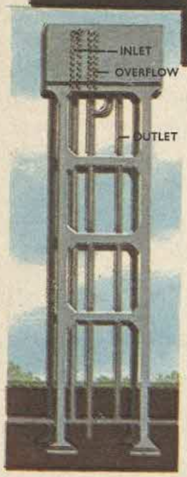


One of Lord Lister's carbolic sprays. Steam generated in the brass container passed out through the nozzle, drawing with it a fine spray of carbolic from the jar.

Lister chose carbolic acid as his antiseptic and first used it in an operation in 1865. He treated not only the wound (a fractured leg) with carbolic but everything that touched it – hands, instruments and dressings. He also used a carbolic spray to kill germs in the air. His 'Antiseptic System' was a success but many people believed that Lister had merely discovered an antiseptic. Lister claimed no such thing – he had not discovered carbolic acid, nor its antiseptic properties. What he had done was to show that wound infections could be cured.

When water is pumped from the river it goes into a large open reservoir. This serves to help out the water supply during dry periods and also plays a valuable part in the purification process, as a large amount of suspended material settles here and many of the disease-producing bacteria will die off. Unfortunately, the conditions in a still body of water are ideal for the growth of minute plants known as *algae.* The plants are removed by filtration, but the filters may require more frequent cleaning if algal growth is heavy. Chemical treatment of the reservoir water may be used to reduce algal growth. Care must be taken at the reservoir to prevent any disturbance of the sediment and to avoid floating debris getting into the filters. At the filtering station the water is allowed to trickle down through layers of sand and gravel. Treatment of the water with chemicals which cause the particles to clump together increases the efficiency of filtration but is not always practical. The first filters are of coarse sand and are easily cleaned by blowing currents of air through them frequently. The water runs through this type of filter at a speed of about twenty feet per hour and the partially filtered water obtained is fed onto the surface of the secondary filters, which consist of gravels covered with about two feet of fine sand. Under the gravels there are porous tiles forming drains through which the water passes on its way to the next stage. These secondary filters are much slower in their action – the water moving at under one foot per hour. This is because the fine sand has much smaller spaces through which the water can flow. The system is, however, preferable



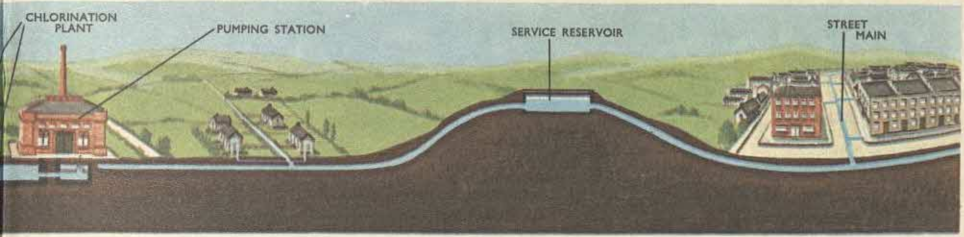


(Left) Cut-away diagram of a water tower showing the inlet and outlet mains. (Above) A simplified layout of a typical waterworks.

to that where only a fine filter was used which was always being choked up with large particles. As the process goes on, a film of fine silt, debris and micro-organisms builds up on the surface of the sand. The bacterial population of this film plays a useful role in that the organisms break down complex organic material into harmless inorganic compounds. The bacteria multiply and form a jelly-like layer round the sand grains in the surface layers. Suspended material is caught in this jelly and provides the bacteria with nutrient material. However, this deposit makes a larger head of water necessary to maintain the required flow through the filter, and eventually it becomes necessary to shut off the water supply and clean the filter by removing the top inch or so of sand and washing it in clean running water. The washed sand can then be replaced and the filter returned to normal use, although its efficiency in removing particles will be somewhat reduced until the film forms again. There are several of these filters at the waterworks so that each can be cleaned in turn without too much hindrance to the purification process as a whole.

Some minor operations may be necessary, according to local water conditions. For example, iron, which would give the water a sour taste and would stain fabric material during washing, must be removed. Other minerals are encountered locally and are removed by chemical processes varying with the nature of the mineral impurity. After removal of all these impurities the water passes to the chlorination plant where it is treated with the gas chlorine. This is the substance used at swimming-pools to kill germs in the water there. At the chlorination plant the water receives a small dose of chlorine which makes sure no undesirable bacteria remain in it. The actual dose applied varies according to local conditions and the quality of the water being processed. However, enough is used to kill off any bacteria, and continuous testing of water passing out of the plant ensures that the chlorine level is not high enough to make the water taste. Although these purifying processes are very efficient, the water is still tested daily for bacteria. A harmless bacterium, *Bacillus coli*, found in everyone's stomach, is very hardy and difficult to kill and has been adopted as an index of purity. If none or only a few of these are present in a test sample it can safely be concluded that there are no harmful bacteria in the water.

Our water is now in a fit state to be used in home and factory. Large pumps, working all the time, pass the water into what is known as the service reservoir. This is a covered chamber where the purified water is stored and from where it passes into the maze of underground pipes that supply the houses in the area. The ideal site for a service reservoir is high up so that the water will build up sufficient pressure to run freely from the main water pipes of the area. In practice, a large town will have a number of these reservoirs, each supplying a certain area. The total volume of water in these reservoirs should be equivalent to the estimated storage need for the whole area for a given period. Small hill-top areas are frequently dominated by a water tower. This is really a small service reservoir built to provide a sufficient head of water for pumping at the pumping station and usually contains about six hours' supply. An arrangement of floats and electrical contacts can be used to start and stop the pumps automatically when the water



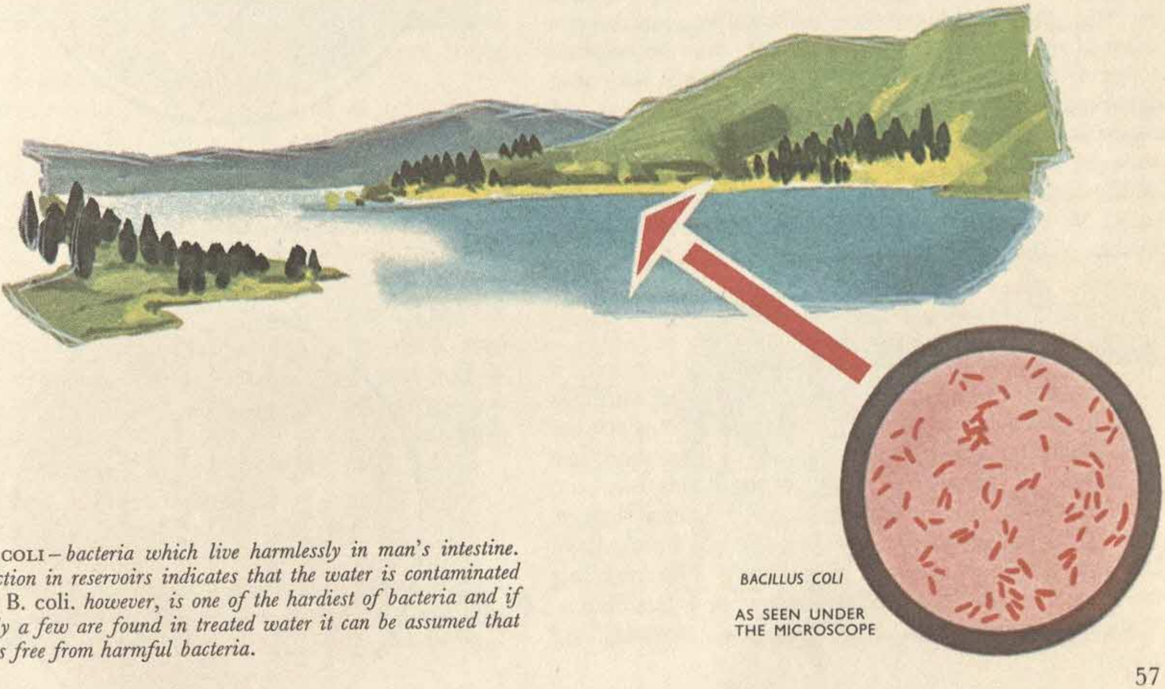
level in the tower falls to a minimum or reaches maximum capacity. The main pipe to the water tower may itself give off branches to the local houses, or there may be a separate outlet main for this purpose. The branch pipes leading to houses have large taps or valves at regular intervals. These are very necessary for tracing leaks and shutting off the water from a small area so that leaks can be repaired or new house pipes joined to the main. Opening the kitchen tap causes the water to flow out of the pipe and down the drain, eventually reaching the river to begin the cycle again.

Bacteriologists at work

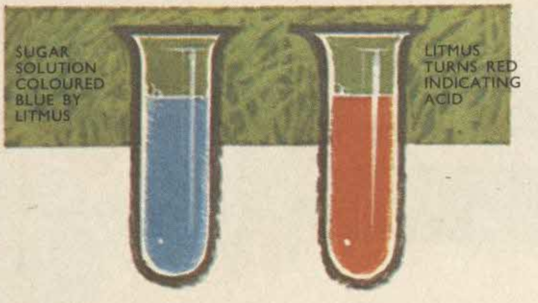
Bacteriology is a specialized division of *microbiology*—a subject which includes the study of all microscopic organisms. Strictly speaking, most bacteriologists should be called microbiologists, for in the course of their work they may also deal with microscopic fungi (moulds) and viruses (organisms so small that they cannot be seen, even with an optical microscope).

Bacteria and medicine

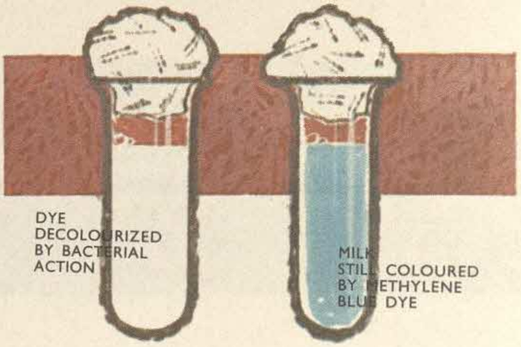
Bacteria, like viruses, cause a variety of unpleasant and dangerous illnesses in man. They are, for instance, responsible for typhoid fever, leprosy, tuberculosis, cholera, diphtheria, tetanus and plague. The bacteriologist is of great help to doctors in combating and preventing such diseases. By the examination under the microscope of blood or other samples taken from the body, he finds the germs responsible for a person's sickness. His identification of the bacteria enables the doctor to prescribe the correct course of treatment.



BACILLUS COLI—*bacteria which live harmlessly in man's intestine. Their detection in reservoirs indicates that the water is contaminated by sewage.* B. coli. *however, is one of the hardiest of bacteria and if none or only a few are found in treated water it can be assumed that the water is free from harmful bacteria.*



Litmus solution shows whether bacteria can ferment certain sugars into acids. Samples of gas given off in the same process are collected in small containers.



Estimating the number of bacteria in milk. The time taken for bacterial action to decolourize the dye, methylene blue, indicates whether the number is above normal or not.

Identification is rarely possible just by examination with the microscope. The bacteriologist usually has to 'cultivate' the germs. He prepares in a dish a mixture of meat extracts and mineral salts (*a medium*) on which they grow. The medium is 'infected' by a few microbes taken from the diseased person and kept at body temperature so that they can grow. They quickly multiply and establish a *culture* or *colony*.

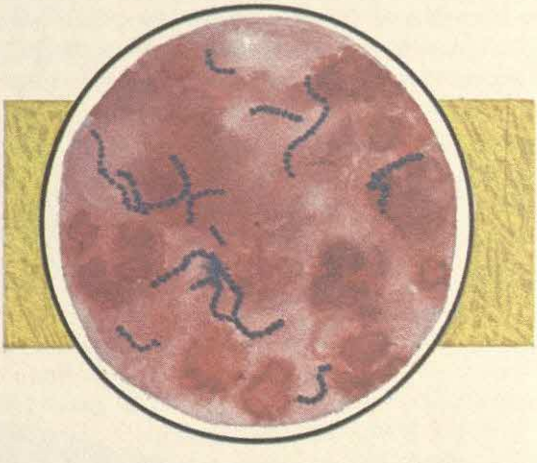
The study of such things as the shape, size, and colour of the colony may be helpful for the bacteriologist in his identification, but more important is the large supply of the pure 'germ' now available for experiments. The bacteriologist can find out what chemical reactions the bacteria cause (e.g. whether they can break down fats, what types of sugar they can ferment). By injection into laboratory animals he can find out exactly their effect on living tissues. Delicate chemical tests are the best means of identifying bacteria, though microscopic examination, and knowledge of symptoms of the sick patient, may be helpful.

Bacteriologists are constantly searching for new chemical compounds to combat bacteria. It is, of course, important that the chemical kills only the bacteria and does no extensive damage to the tissues of the sick person. Disinfectants and antiseptics are easier to find; they are chemicals used only on the surface of the body.

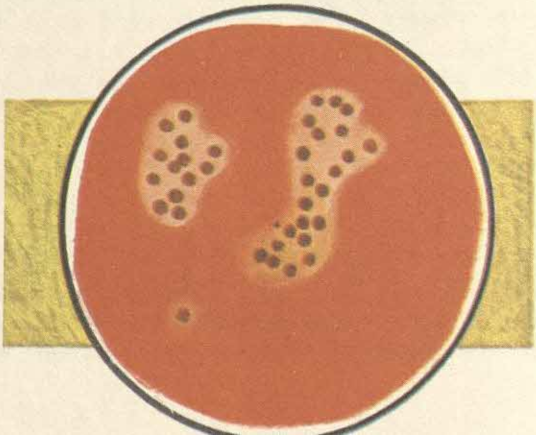
Bacteria and public health

Harmful, disease-causing bacteria live and multiply in filth, and so great care is taken to dispose of sewage and garbage. Sewage is decomposed into harmless substances by the use of certain oxygen-loving bacteria. The process is supervised by trained bacteriologists. The same microbes are also being used to break down the dead organic matter in rubbish. The resulting material from the process can be used as a fertilizer.

Bacteriologists also supervise the storage and treatment of public water supplies. If a reservoir serving a large town were to become contaminated with harmful bacteria a large-scale outbreak of disease – an epidemic – would follow. The water thus has to be continually examined for its bacterial content. As it is very difficult to detect harmful bacteria



STREPTOCOCCI *appear under the microscope as chains* (*above*). *The harmful forms which cause disease can haemolyse blood* (*i.e. damage red blood corpuscles allowing the red haemoglobin to escape*). *This test can be used to distinguish them* (*below*).

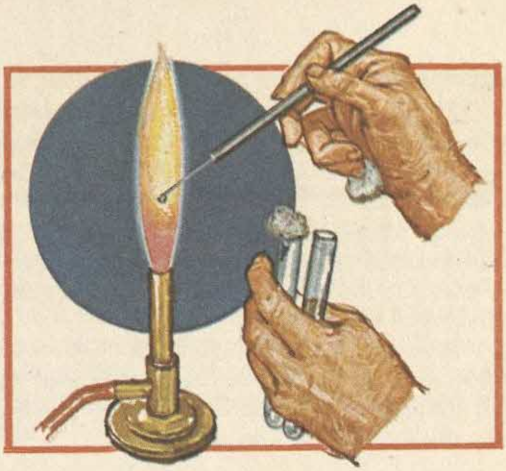


by mere inspection, the bacteriologist makes cultures of the overall bacterial population taken from a sample of water. From the cultures an estimate of the number of bacteria in the whole reservoir can be made. If there are more bacteria than usual, the bacteriologist will immediately suspect contamination. Contamination of water supplies is nearly always caused by seepage of sewage and excreta into wells and reservoirs. When this happens, a certain type of *coliform* bacteria becomes present in the water. Detection of this microbe in the cultures immediately indicates to the bacteriologist that recent pollution has taken place and water supplies must be stopped until the water is made safe. Water in public swimming-baths undergoes similar routine checks.

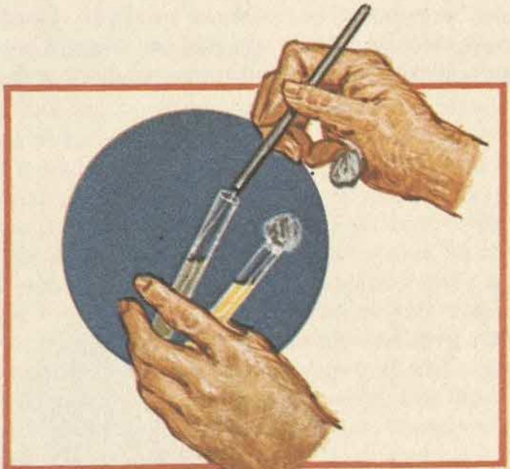
An outbreak of disease may be caused by contamination of food supplies. 'Canned' and pre-packed foods may not be used until a long time after they have been sealed; it is therefore important that they are prepared and packed under sterile conditions supervised by bacteriologists. Canteens and slaughter-houses are regularly inspected for cleanliness, and imported foods, particularly meat and eggs, are examined by bacteriologists for presence of harmful microbes.

Great precautions are taken in this country with milk. It is a food used in large quantities, especially by babies and children. The numerous processes milk has to undergo, from the milking of a cow to the opening of a milk bottle, give great opportunity for bacteria to contaminate it. Some infections may be caused by the handlers of the milk, and so bacteriologists ensure no people with contagious diseases are employed in dairy work. In the past, scarlet fever, diphtheria, tuberculosis and sore throats were transmitted by people working with milk.

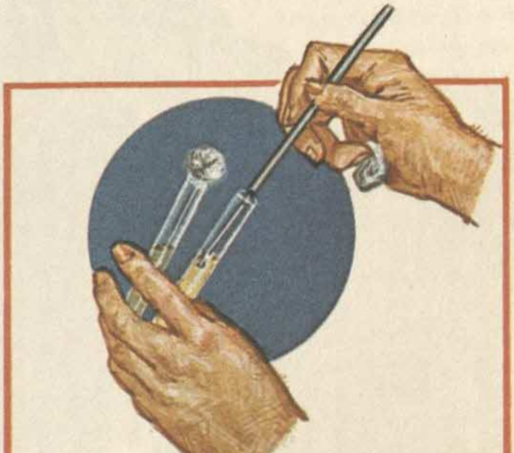
More frequently, harmful bacteria in milk came from the cow itself. Tuberculosis and food poisoning are important diseases which these microbes can cause. Inspection of cows has largely removed the danger of tuberculosis, but milk is still tuberculin-tested to ensure it is free from bacteria causing this disease. Pasteurisation of milk kills the harmful bacteria and most other organisms in the milk without changing the milk's properties. Pasteurisation is not, however, used to make contaminated milk fit for human consumption – it is merely an additional safeguard for milk which has already been tested and is mainly concerned with prolonging the keeping period of milk.



Infecting a liquid medium. The cotton-wool plugs belonging to the test-tubes must not be placed on the bench for fear of contamination. The platinum loop is sterilized in a bunsen flame.



The plug is removed from the tube containing bacteria and the cooled loop inserted. A thin film of liquid carrying the bacteria forms inside the loop.



The loop is passed below the surface of the prepared liquid medium and rubbed gently against the side of the tube to dislodge the bacteria. The cotton-wool plugs are replaced.

Immunity

There are some diseases which are characteristic of childhood. In many cases, once a child has suffered from German measles it is unlikely to get a further attack throughout life. It seems that the disease causes some change in the body which enables it to resist further invasions by the disease-causing organisms. In this case the individual is said to be immune – to have developed immunity.

The history of the investigations which have led to the practical measures which have eradicated smallpox and almost conquered poliomyelitis in Britain starts with the observations of Edward Jenner.

Edward Jenner, M.D.

One of Edward Jenner's early interests was the means of preventing outbreaks of smallpox. Smallpox in the eighteenth century was still one of man's worse scourges. Periodically, epidemics would break out killing thousands of people. Local tradition in Gloucestershire, Jenner's county of birth, had it that those people who suffered from cowpox – a mild and harmless disease – would remain unaffected by the fatal smallpox. There were many stories to support the tradition.

In 1775 he began to document all the relevant case-histories known. Unfortunately, about this time occurrences of cowpox were rare in Gloucestershire, and Jenner's theoretical work could not be supplemented by either first-hand observations or experiment.

Cowpox returned in 1796 and Jenner acted promptly. Material from a cowpox vesicle on the hand of a local dairymaid was taken and introduced into the bloodstream of an eight-year-old boy – one James Phipps. Two months later Jenner performed the crucial experiment. He injected virulent smallpox material into the boy. And the boy remained well. The experiment was repeated – again with success – and in 1798 Jenner wrote a memorable paper on the subject.

One of man's greatest benefactors, Edward Jenner. By his work, smallpox was conquered and the way was cleared for the prevention of many other diseases.

Chance injections had occasionally been tried in the past, but Jenner's careful work put the subject of inoculation against smallpox onto a firm scientific basis. A stage had been reached when an all-out crusade against the disease could begin.

However, Jenner's work was subject to criticism – not always well informed. Returning to London, he prepared his own material and inoculated thousands of poor people free of charge. Significant drops in the fatality of smallpox immediately followed. The tide turned in Jenner's favour and in 1802 he received the first of his grants from the British Government. In the following year the Jennerian Society was founded to promote wide-scale inoculation against smallpox. The society flourished until 1808, when the National Inoculation Establishment was founded. The principle of vaccination was eventually to lead to the immunization of man against other dangerous diseases.

Vaccines

The human body is constantly subject to attack and invasion by disease-producing bacteria. Fortunately it has built-in mechanisms for repelling and suppressing the germs and neutralizing the poisonous substances (*toxins*) that they produce. It is not naturally immune to all infectious diseases, however, but if attacked, and if it survives, it will often be immune to a subsequent attack because *antibodies* have been produced by the body in response to the first attack. These may only remain in the blood for a short time, but the ability of the body to produce the necessary antibodies quickly is greatly enhanced.

Immunity may be acquired artificially by *inoculation* or *vaccination*. This entails giving the body a mild dose of the disease so that it produces antibodies. The substance used is called a *vaccine*. It is usually prepared from the weakened or killed organisms that cause a certain disease, or from the toxins they produce. Alternatively, related but harmless organisms that produce some toxins similar to those of the disease-causing forms are used.

This may produce very slight symptoms of the disease but is sufficient to provoke the necessary response by the body.

Each type of antibody is specific for a bacterium (or its toxins) producing a particular disease. Thus separate vaccines have to be administered in order to stimulate the production of antibodies for several diseases. Combined vaccines have been developed in order to reduce the number of injections needed. They are commonly used for the vaccination of infants. An example is the combined vaccine for whooping-cough, tetanus and diphtheria.

The production of a vaccine is a most complicated process, a large part of which is concerned with ensuring that each batch of vaccines is safe, the most stringent tests being carried out. Basically, the process involves the culture (growing) of the bacteria or viruses concerned on a special medium – the *culture medium*.

Viruses have to be cultured in living media; they can only grow and reproduce in living cells. Hens' eggs make ideal environments for the culture of viruses – the virus causing fowl-pest is cultured in such a medium. Bacteria are grown on non-living media – usually liquid broths contained in large fermentation vessels.

Polio vaccine

As different viruses require particular cells in which they can grow, the selection of the correct tissue for culture-bottle preparation is extremely important. Polio virus grows best in healthy monkey kidney tissue. The Salk polio vaccine, developed in America by Dr. Jonas Salk, is a killed vaccine. The cultures are held at a temperature of 37°C. for seven days in a special medium designed to maintain a good growth of cells. When the single-layered sheet of cells covers the inner surface of the culture bottle the spent fluid is changed and replaced with fresh fluid, together with a small amount of the living virus. Cultures are reincubated for three to four days, and during that time the virus will enter the cells, grow rapidly in them and ultimately destroy them. Finally, the virus, which has now multiplied many thousands of times, is harvested in the fluid medium. The virus fluid now forms the basis of the vaccine. The medium contains very small quantities of antibiotics in order to prevent the growth of foreign bacteria and moulds. When the virus fluid has passed a series of stringent tests it is ready for further processing. It is filtered to remove cell debris and virus clumps. The filters are extremely fine and allow the passage only of individual virus particles. This is extremely important because the next stage is the addition of the killing agent, formalin, and it must be able to attack and kill each and every virus particle to ensure that the vaccine is free from infectious virus. Large samples are now removed from the bulk single-strain pools for safety testing in monkey kidney cell cultures to make sure that no live virus remains in the vaccine. If a live virus were present it would destroy the cell sheet and thus be detected. It is interesting to note that as many as a thousand cultures are used to test each single-strain pool and that it takes at least six months to prepare.

Poliomyelitis is caused by three distinct viruses, and for the vaccine to give complete protection

Fertile hens' eggs are inoculated with virus, one of the first stages in the production of fowl-pest vaccine. Two research workers harvest virus by taking fluid from a number of eggs.

A technician 'refeeding' tissue cultures with fresh nutrient medium, a stage in the safety testing of Polivirin, the poliomyelitis vaccine.

A late stage in the manufacture of Polivirin. The pools of single-strain vaccine are blended in these large stainless steel containers.

against this disease it must contain all three types of virus. Each type of virus is grown and processed separately. After the pools have passed their safety tests satisfactorily they are then blended together to form a *trivalent* vaccine. Repeat safety tests are done on the final vaccine in tissue culture, and in addition monkeys, which are highly sensitive to poliomyelitis and receive cortisone to increase this sensitivity, are inoculated with the vaccine and watched for eighteen days to see if they remain healthy.

The most stringent safety precautions are taken during the final stages of production. Only gowned and masked operators handle the vaccine. When the 'pools' arrive at the unit concerned they are transferred aseptically through a special panel into similar containers, thus eliminating the chance of contamination being introduced into the blender-room. Access to and from this room is made via a shower-bath. Samples for the tissue-culture safety tests are taken at this stage.

The pools of single-strain vaccines are transferred in equal volumes to 450-litre stainless-steel vessels. These are rotated for two hours to achieve complete mixing. The final vaccine, called *Polivirin*, is usually amber in colour.

'Living' poliomyelitis vaccines, i.e. those consisting of living but weakened germs, are given orally on sugar lumps, whereas 'killed' vaccines have to be injected.

Fowl-pest vaccine

Fowl-pest is a highly infectious disease affecting all types of poultry. Since the Second World War, in particular, the disease has been the cause of heavy financial loss to the poultry industry the world over. Where outbreaks of the disease occur the practice is to slaughter all birds.

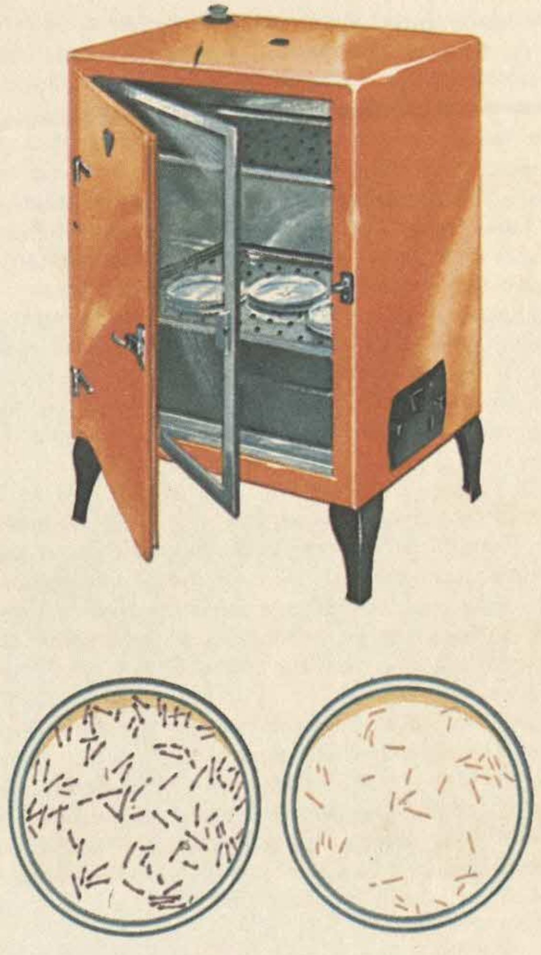
With the great development of the broiler industry disease spreads readily and the colossal destruction of birds is becoming more and more uneconomic, so that the need for a vaccine is a great one. This need has probably now been satisfied by the production of a killed vaccine, one injection of which gives prolonged immunity.

The virus – a strain of Newcastle disease virus – is injected into a cavity in fertile hens' eggs. The eggs are incubated for a certain length of time during which the virus multiplies. It is harvested, by extracting the fluids from within the eggs, inactivated and then stored at a temperature of 4°C. whilst samples undergo vigorous testing to ensure that the vaccine is safe but sufficiently potent. After testing, batches of the vaccine are blended with aluminium hydroxide to increase the potency further. A 'buffer' is added to stabilize it, and then a preservative.

Extensive safety tests are carried out with the vaccine on growing embryo chicks (those still in the egg) and on young chicks.

If stored under suitably cool conditions (i.e. at a temperature between 2°C. and 10°C.) it will not lose its potency for twelve months. Under warmer conditions it does not remain stable for such long periods (e.g. at 37°C. it loses stability within seven days).

The design and production of a new type of syringe, shaped like a gun and easily operated by hand, has eased the problem of giving vaccine to large numbers of birds. Young birds are best injected in the breast muscle, but older chickens and turkeys may be injected in the neck or thigh.



The appearance of bacteria stained by Gram's method. (Left) Gram-positive organisms that have been stained purple with methyl violet. (Right) Gram-negative organisms that have accepted only the counter-stain. (Top) An incubator used for culturing bacteria under carefully controlled conditions.

Identifying the infective organism

When a person does become ill because of the effects of a pathogen various routine procedures are operated. Samples of the pathogen have to be obtained, and these, together with information on the state of the patient, his case-history, the length of his illness, the origin of the samples and so on, are passed to the bacteriologist whose job it is to identify the infected organism. Once this has been established the doctor can then prescribe more precise treatment.

Samples of the pathogens are obtained from infected material in a variety of ways, depending on the site of infection. It is important that they are collected either before the patient has received any antibiotics or immediately before the next dose is given.

Swab sticks are commonly used to obtain samples from the nose, throat and ears, and from wounds. They consist of thin wooden sticks round one end of which is wound a small piece of sterile (germ-free) cotton-wool. They are kept in sterile tubes. In taking throat swabs great care must be taken to avoid contaminating the swab with the organisms that normally live in the mouth. The swab must only touch the swollen tonsils. In taking nose swabs care must be taken to avoid damaging the delicate tissue lining the nostrils. The nose and throat swabs are returned to their sterile tubes and sent quickly to the bacteriological laboratory. Sterile containers need not be used for the collection of sputum (mucus) from the lungs, since it is always contaminated by other organisms in the air (though the containers must be clean).

In taking blood samples it is essential that the necessary measures are taken to ensure sterile conditions. Most pathogens are likely to be present in the blood when the patient's temperature reaches its highest level, and it is important that the sample is taken at this time. A dry sterile syringe is used.

Some specimens (so-called *puncture fluids*) are obtained by inserting a sterile syringe into a cavity in the body. Samples of cerebrospinal fluid and fluid in the chest cavity are obtained in this way. They must be placed into sterile tubes to avoid contamination.

The bacteriologist has a number of tests at his disposal by means of which he can identify the pathogen. These include its size and shape and how it takes or rejects certain stains; how it grows – which substances and conditions encourage or inhibit growth; its resistance to heat, drying, antiseptics, etc.; its reaction to specific antibodies, and the effect of infected material on laboratory animals.

The shape of bacteria can be seen under the microscope, but to make them more clearly visible they are stained, using special dyes, by a technique called *Gram's method*. The bacteria are first stained with a basic dye, methyl violet, and then treated with iodine. Unwanted stain is removed by washing in alcohol and then they are stained with a dye of contrasting colour – a process called *counter-staining*.

Bacteria that retain the first dye and are purple in colour are said to be *Gram positive*. Those that accept the counter-stain are said to be *Gram negative*. Gram's method is thus a way of dividing the bacteria into two groups. There are some bacteria that do not accept Gram's stain very readily and other methods have to be employed. However, those that can be classified as Gram negative or Gram positive, together with their shape, are part way towards identification.

In order to separate an organism from the others with it (a sample will generally contain non-pathogenic bacteria as well) the usual practice is to place them on the surface of a jelly, *agar-agar*, which has had food material (e.g. meat broth) added to it (the food is called a *culture medium*). By selecting the medium it is possible to prevent the growth of certain organisms. This is a way of confirming the identity of a particular bacterium. Selective media are known for most pathogens.

Having isolated a type of bacterium, the conditions affecting growth are investigated if further identification is needed. They are cultured in incubators at body temperature (this is the most favourable temperature for the growth of most human pathogens). The atmospheres in which they grow may be adjusted and they are left for up to twenty-four hours. The extent of their growth, shape, size and so on is

Some present-day hospital practices

Both in wards and in the operating theatre every attempt must be made to reduce to a minimum the chances of infection spreading. Thus any contaminated bedclothes, mattresses and so on, must be sterilized by placing them in units through which steam is blown under pressure. Nursing staff must frequently wash their hands, masks must be worn when preparing treatment trolleys, wards must be ventilated efficiently and the amount of dust liberated during bed-making must be kept to a minimum since dust is a rich source of organisms. Waste materials – soiled dressings, etc. – must be disposed of by burning in incinerators, and faeces must be disinfected.

In the operating theatres the walls and the floors are frequently washed down, special newly washed clothing must be worn by all entering the theatre; the circulating air is filtered to remove dust, warmed and moistened; the surgeon and his assistants wear masks and sterile gloves; and, of course, all instruments must be sterilized.

In some hospitals there are special germ-free wards where the condition of patients is such that on no account must they come into contact with pathogenic organisms. Such is the case when organs are transferred from one person to another unrelated person. Normally the body will reject tissues that are foreign to it. By irradiating the tissues (with X-rays or γ-rays) their ability to reject the foreign organ is so reduced that the natural reaction to it is not set up. But the irradiation also reduces the ability of the body to produce antibodies so that, until the transplanted organ has 'taken' successfully, sterile conditions must be maintained. Food is irradiated and nursing staff wear sterile clothing and masks when entering the patient's room. Ultra-violet lights in the room also kill many bacteria.

important in their identification. The organism that causes tetanus, for example, is unable to grow in the presence of free oxygen. It must be cultured in an environment from which oxygen has been removed.

Certain bacteria possess an enzyme that enables them to bring about a specific chemical reaction. The ability of one of two closely related organisms to break down a carbohydrate, for example, is a means of distinguishing between them. Thus, the diphtheria-causing organism is the only species of its genus that is able to break down starch.

Curing disease

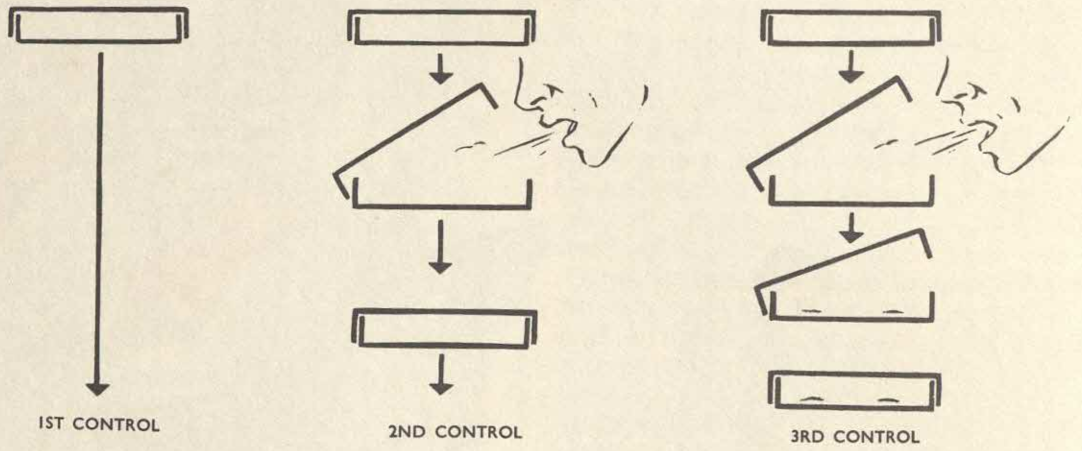
When a bacterium or virus has become established in the body two courses of action may be taken. The first is to eliminate the symptoms – to make the patient as comfortable as possible, for example by pain-killing drugs such as aspirin. The second is to attack the organism causing the disease. Perhaps a third point which should be mentioned is to take account of the symptoms, which makes diagnosis possible (which may mean identification of the cause) and helps in prescribing treatment.

The search for substances which kill bacteria and viruses but leave human tissues undamaged has been long and arduous.

Try to imagine the problem of knowing where to start. Bacteria are living organisms, they are affected by poisons which affect most living things. To try systematically to find the effect of all known substances on bacteria would be an impossible task. Paul Ehrlich (1854–1915) made a very interesting observation – that certain dyes were taken up by bacteria but not by the tissues in which they were found. He reasoned that if a poisonous substance could be found which behaved like these dyes towards bacteria but was not taken up by human tissues it might be possible to attack the bacterium when it was in the body. After testing 605 substances Ehrlich discovered his '606' or saluansan. In this way a step was taken along the road which led to the development of such modern drugs as the sulphonamides.

Setting up trials of each possible substance in investigations of this kind is a complicated and exacting task. Progress is usually made in little stabs.

Against this background, the discovery of the effect of the fungus Penicillium on the growth of certain bacteria by Professor A. Fleming in 1928 was a dramatic breakthrough.



Practical

Set up agar plates as described on p. 21, treated as follows:

a. Not exposed (1st control).

b. Exposed briefly, cough on the plate (2nd control).

c. As for (b) but with three penicillin discs* placed on the agar after coughing on the plate.

* Penicillin discs can be obtained from OXOID Ltd., Southwark Bridge Rd., London, S.E.1.

N.B. Culture at room temperature for a week.

QUESTIONS

(1) What is the purpose of the 1st control?

(2) Does it matter if different people cough on plates (b) and (c)?

(3) Are any bacteria which you have cultured resistant to penicillin? Or sensitive to penicillin? How do you know?

Fleming and his discovery of penicillin

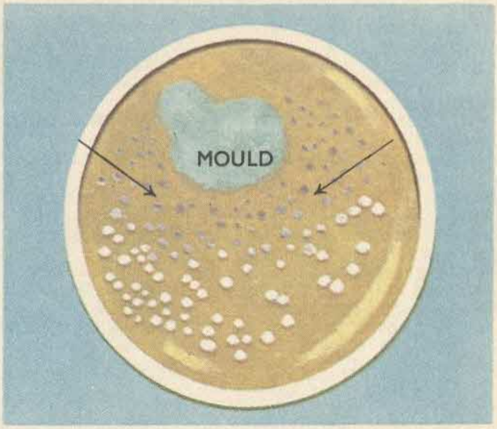
The discovery of penicillin and its subsequent development will always be among the most outstanding advances ever made in medicine, yet the original discovery stems from an accident – a chance happening in a London laboratory.

In 1928 Professor (later Sir) Alexander Fleming was working at St. Mary's Hospital on problems associated with disease-causing bacteria. One day, when inspecting cultures of *Staphylococcus* that he had grown on agar, he noticed a green mould growing in one of the dishes. In the normal course of events such a contaminated dish would have been thrown out, but Fleming examined it more closely and made a very important discovery. In the neighbourhood of the mould there were no thriving colonies of bacteria. Fleming reasoned that the mould (later identified as *Penicillium notatum*) was producing a substance able to kill bacteria, and that it could be important in the medical field. After photographing the dish, he removed some of the mould and began to experiment.

When he grew it in a liquid medium, Fleming found that the liquid he could filter off was active against a number of bacteria, including those responsible for boils, pneumonia and meningitis. This crude liquid Fleming called 'penicillin'.

Although he was successful in treating many wounds with such a liquid, he realized that for large-scale use it would need to be purified and concentrated. Early attempts to do this failed because, although the liquid was stable at low temperatures, it broke down during the treatments used. In 1932 the work was abandoned.

In 1939, at Oxford University, Professor Howard Florey and a team of chemists headed by Dr. Ernst Chain again took up the problem. They succeeded in obtaining first a watery solution and then a dry yellow powder, both many times more powerful than Fleming's original culture liquid. Although it is now known that the powder contained about 99% impurity, encouraging experimental results were obtained with it. The results were good enough to justify commercial production. Because of the Second World War resources were not available in Britain, and as a result of Florey's visit to America the first large-scale production was in the United States.



This reproduction of Fleming's original picture shows the dying bacterial colonies (arrowed) in the region of the green Penicillium *mould.*

Penicillin saved many lives during the later stages of the war, and since 1946 it has been generally available as a valuable aid in combating disease. Over the years great progress has been made in the production of penicillin, and now an extremely pure form can be produced. It is active against species of bacteria at doses as low as one part in sixty million parts of solution. For their work with antibiotics, and with penicillin in particular, Fleming, Florey and Chain shared the 1945 Nobel Prize for Medicine.

Treatment with serum

In countries where scorpions or poisonous snakes are common stocks of serum or anti-toxin are available for treating victims. Similar sera are available for treating certain bacterial infections, such as tetanus. The sera are produced by injecting horses with the poison concerned in quantities large enough to cause anti-body formation but not large enough to harm the horses. Blood is later taken from the horses and the sera are extracted and kept until needed. Injections of the appropriate serum will destroy poisons already in the body and cure the affected person but the injections do not give any protection against further snake-bites or infections.

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